GLYCOMATERIALS

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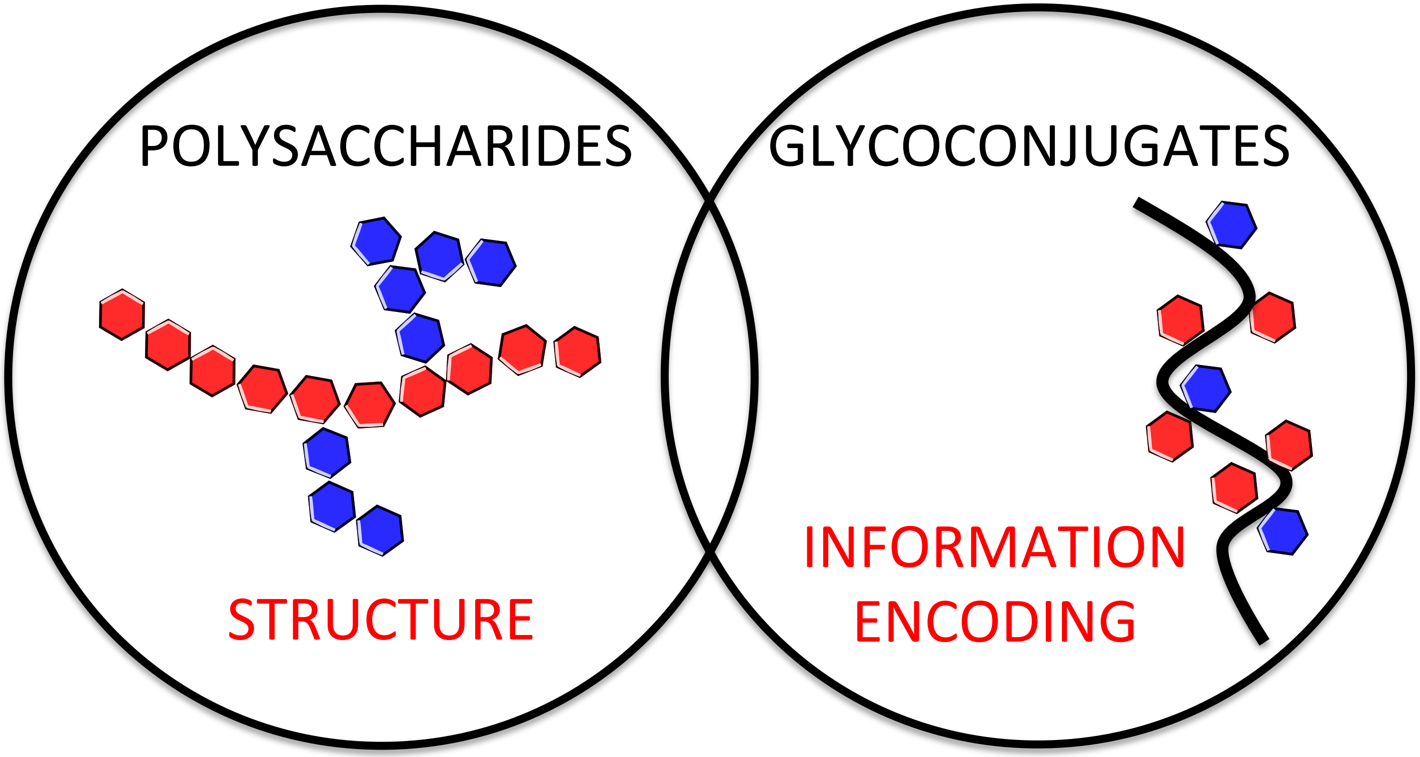
UCSD Chemistry and Biochemistry

Compelling reasons to stay away from sugars:

- Glycans are metabolic products without direct link to DNA
- Glycans are compositionally and structurally complex
- Glycans are difficult to isolated in pure form
- Glycans cannot be amplified
- Glycans are difficult to sequence
- Glycobiology is a nanoscale problem

The nanoscale dimension of "glycobiology"

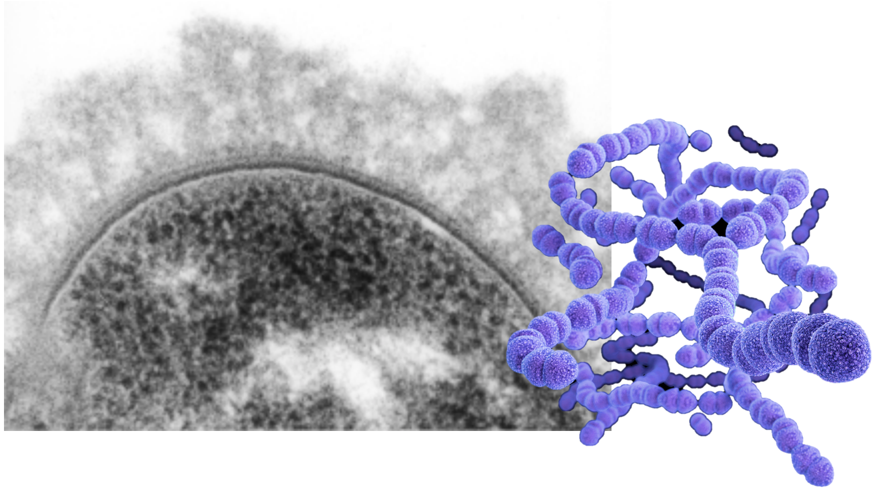
- Carbohydrates (or glycans) exert their functions over length scales far beyond those of their individual building blocks.



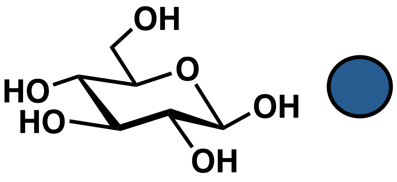
- The study of glycobiology necessitates material science approaches

Part I. Polysaccharides – structural motifs that shape life

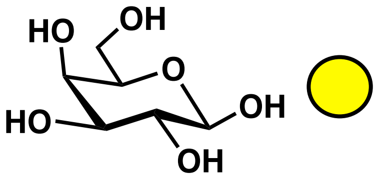
Polysaccharides: Nature's structural materials



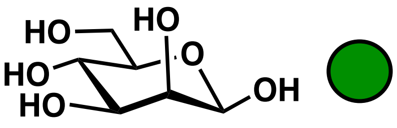
The basic building blocks of polysaccharides



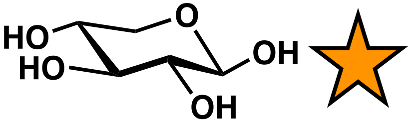
D-Glucose (Glc)



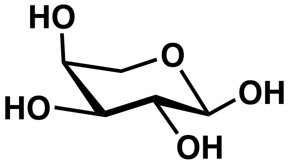
D-Galactose (Gal)



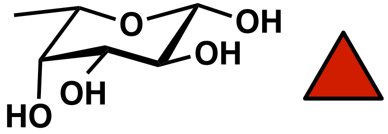
D-Mannose (Man)



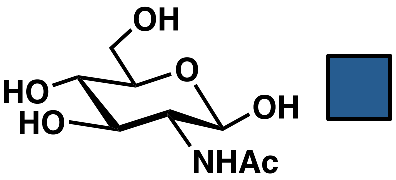
D-Xylose (Xyl)



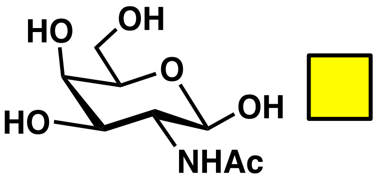
D-Arabinose (Ara)



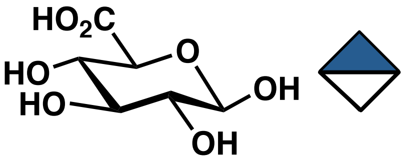
L-Fucose (Fuc)



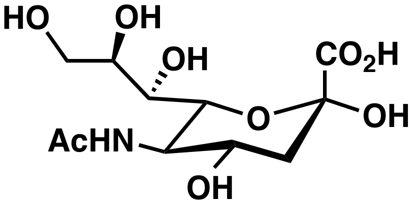
D-*N*-Acetylglucosamine
(GlcNAc)



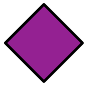
D-*N*-Acetylgalactosamine
(GlcNAc)

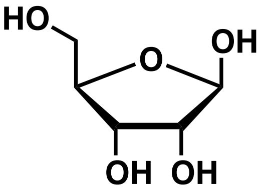


D-Glucuronic Acid
(GlcA)



N-Acetylneuraminic Acid
(Neu5Ac)
or Sialic acid (Sia)

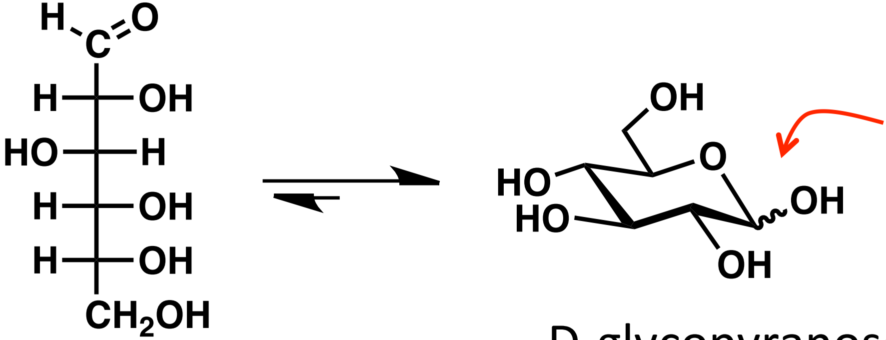




D-Ribose

The bonds that hold sugars together

aldehyde
function

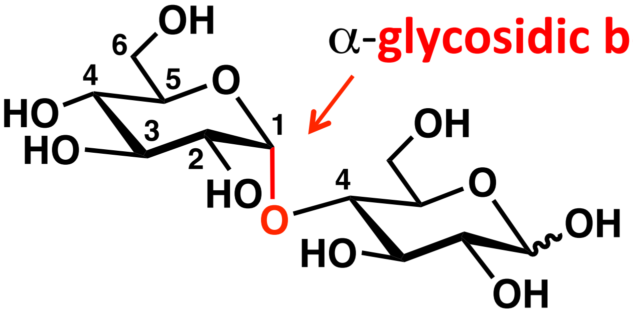


hemiacetal
function
“reducing
end”

D-Glucose (Glc)

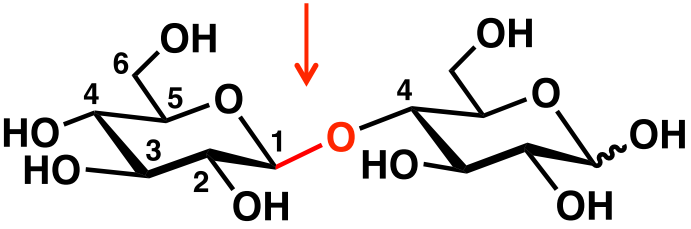
D-glycopyranose
form

α-**glycosidic bond**



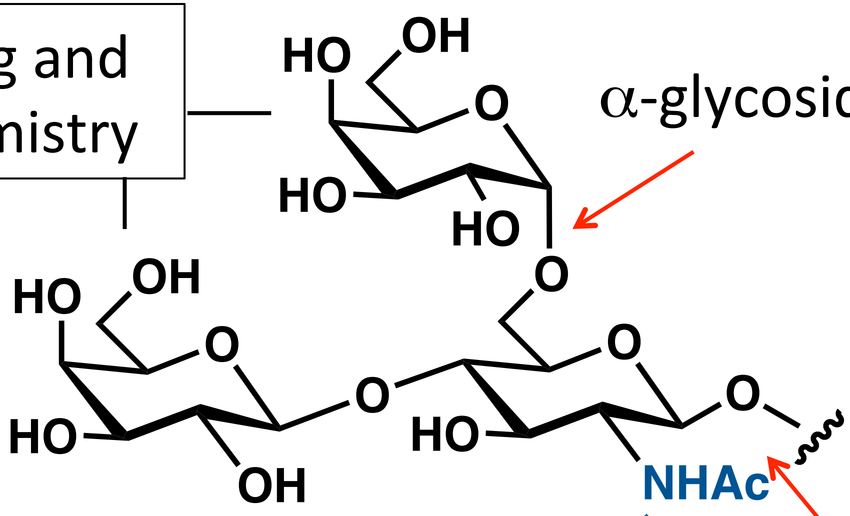
α-glucopyranosyl-(1,4)-glucopyranoside
Glcα1-4Glc, maltobiose

β-glycosidic bond



β-glucopyranosyl-(1,4)-glucopyranoside
Glcβ1-4Glc, cellobiose

Branching out: it's a wild world out there



branching and regiochemistry

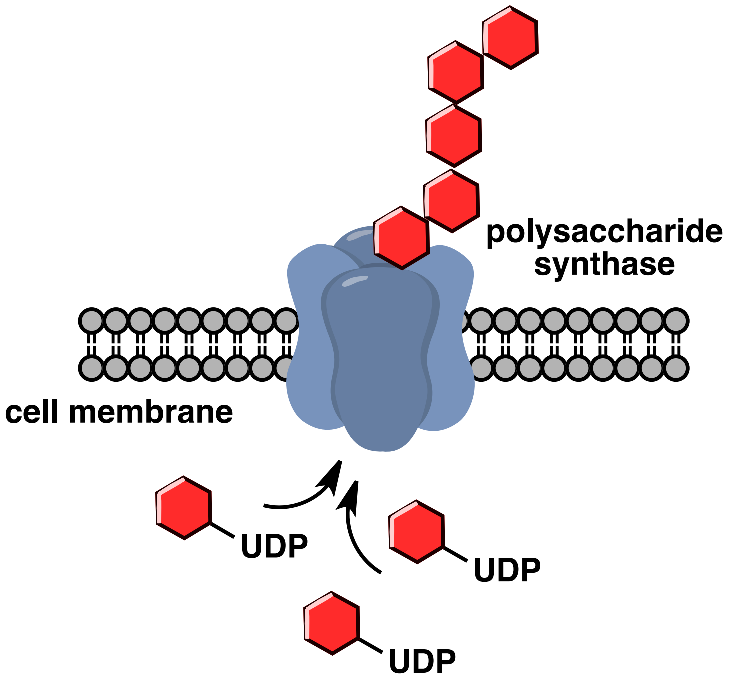
α-glycosidic bond

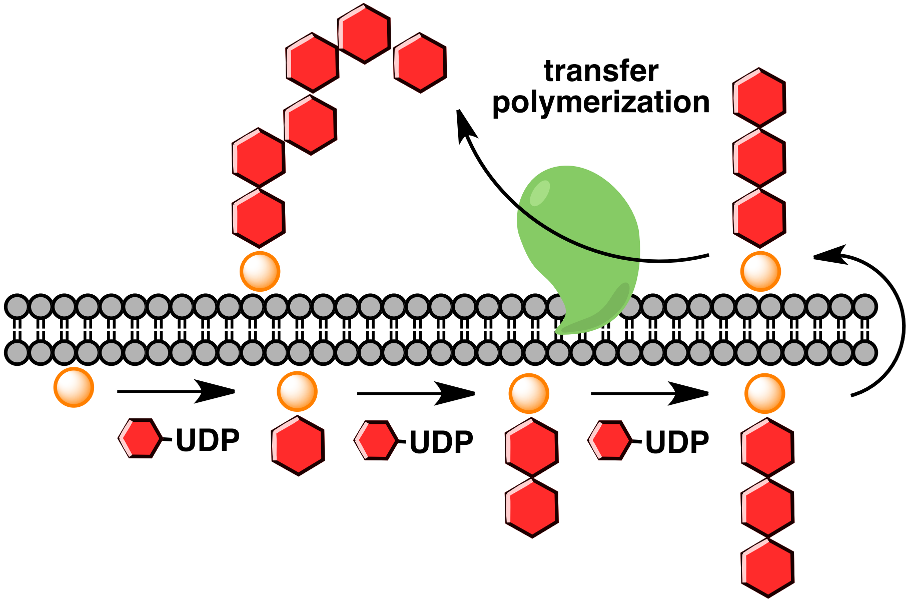
stereochemical diversity

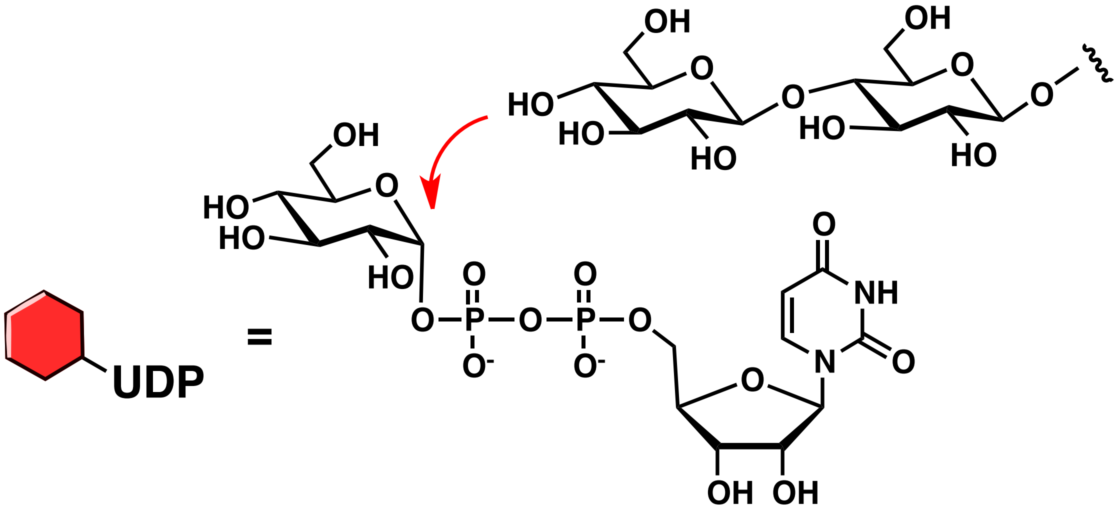
β-glycosidic bond

functional group diversity

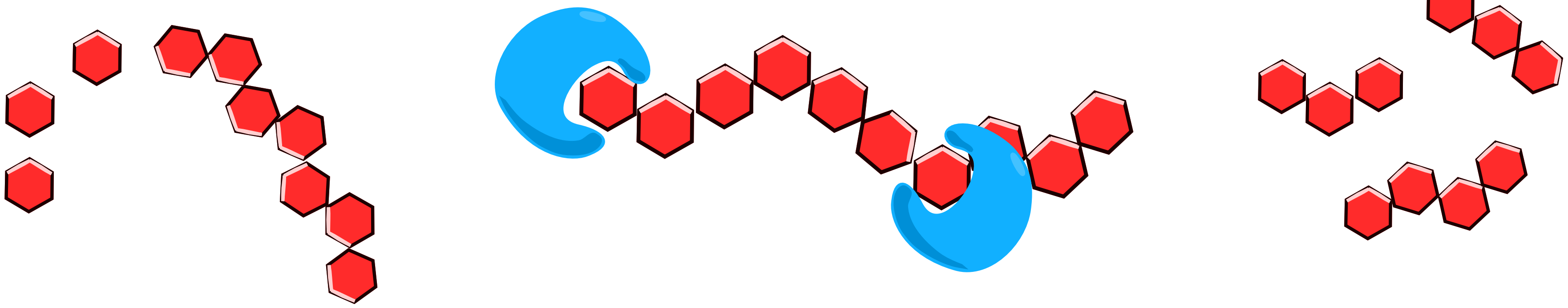
How does Nature make polysaccharides?





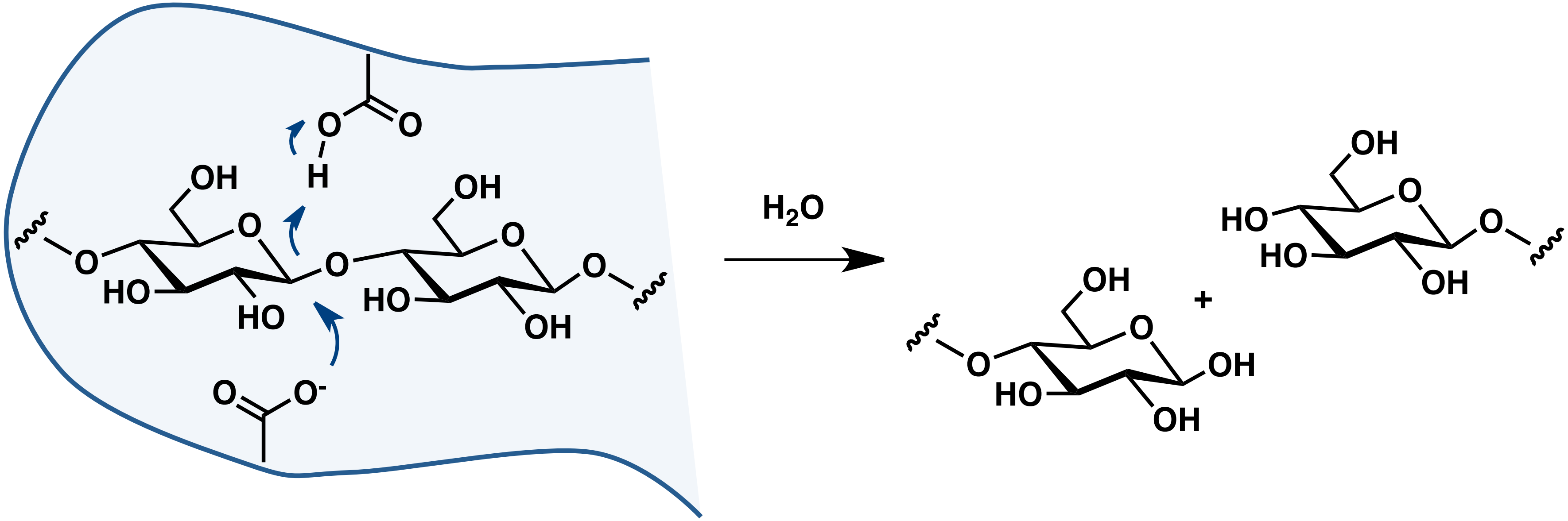


How does Nature break polysaccharides?



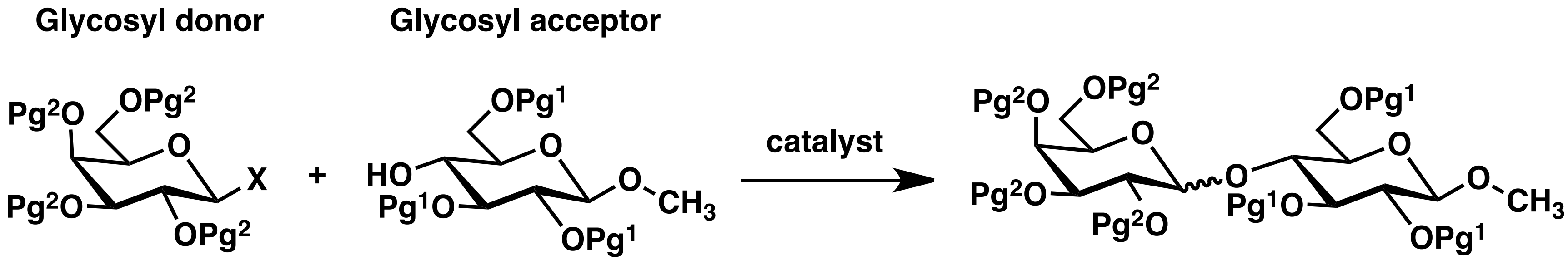
exoglycosidase

endoglycosidase



How do people make polysaccharides?

- de novo chemical synthesis is challenging due to lack of chemo-, regio- and stereoselectivity of glycosidic bond formation:

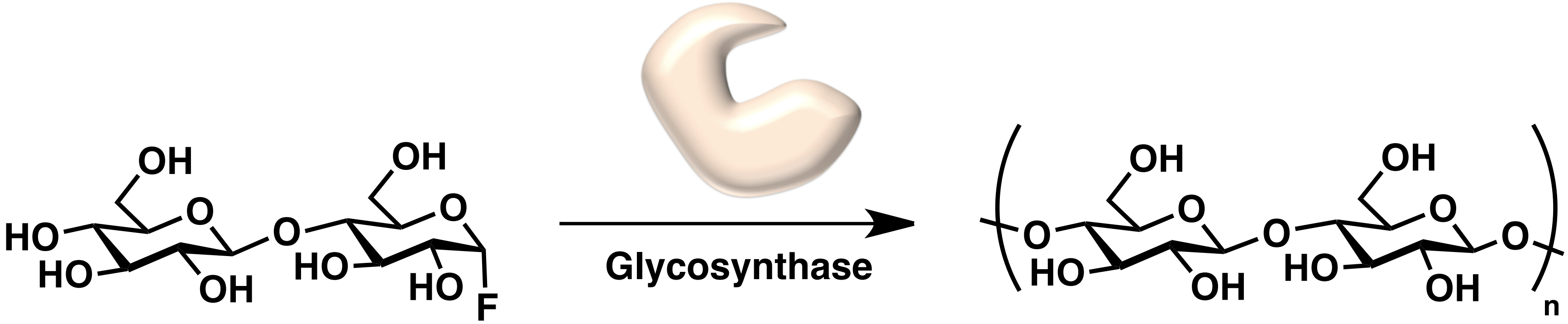


$Pg^{1,2}$ = protective groups
X = activating group

- Isolation from natural sources via partial hydrolysis (acidic conditions)

- chemoenzymatic synthesis (running glycosidases in reverse or with engineered glycosynthases)

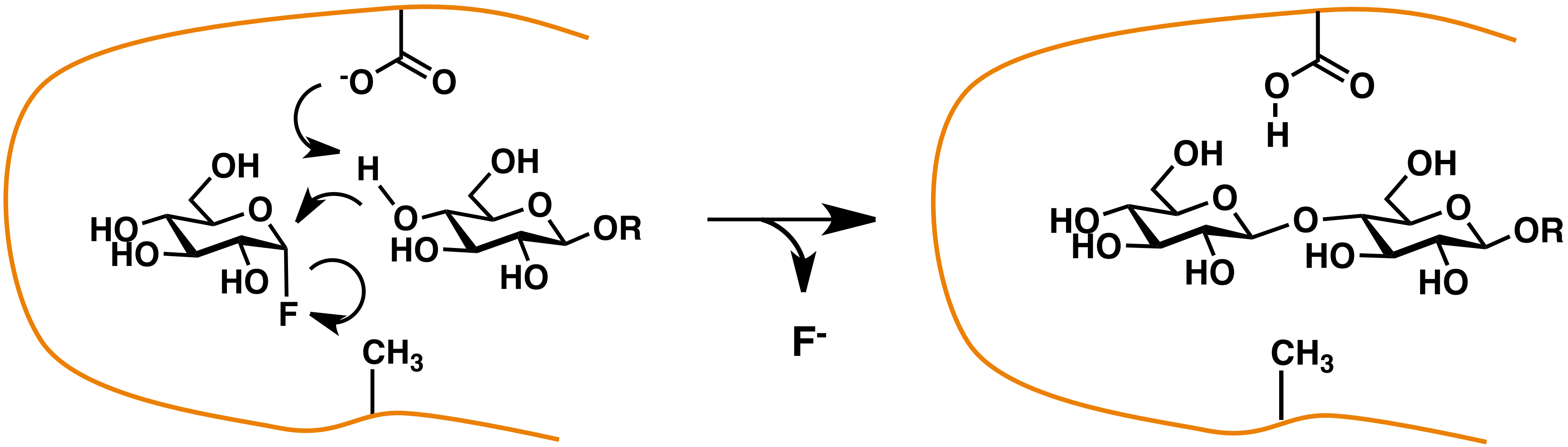
Polysaccharide synthesis using glycosynthases



Cel7B mutant of β-glucosidase from *Agrobacterium sp.*

cellulose

broad Mw distributions



Mackenzie, Wang, Warren and Withers *JACS 120,* 5583 (1998)

Characterization of polysaccharides: challenges

- Structure of polysaccharides is not encoded by genes.
- Polysaccharides are compositionally complex and often branched, polydisperse, high molecular weight polymers.
- Polysaccharide chains can be partially organized but are mostly amorphous.
- Polysaccharides form higher order supramolecular assemblies (semi crystalline or amorphous aggregates, gels, etc.).

Characterization of polysaccharides: methods

- Composition:

  IR spectroscopy (identification of functionality , e.g., amido or carboxy groups

  Chemical or enzymatic hydrolysis to monosaccharide building blocks followed

  by LC/MS or MS analysis.

- Size:

  Size exclusion chromatography or gel electrophoresis

  Sedimentation analysis

  Light scattering

Characterization of polysaccharides: methods

- Molecular structure (atom connectivity, anomeric configuration, conformation):

  Nuclear magnetic resonance (^{1}H and ^{13}C NMR, COSY, NOSY)

  X-ray and neutron diffraction of simple well defined oligosaccharides

- Supramolecular structure and interactions (crystal packing, hydrogen bonding):

  Small angle x-ray scattering

  Small angle neutron scattering

  Solid state NMR techniques

  AFM and EM imaging

  Computational approaches (molecular dynamics)

Polysaccharide classes to be discussed

- NEUTRAL

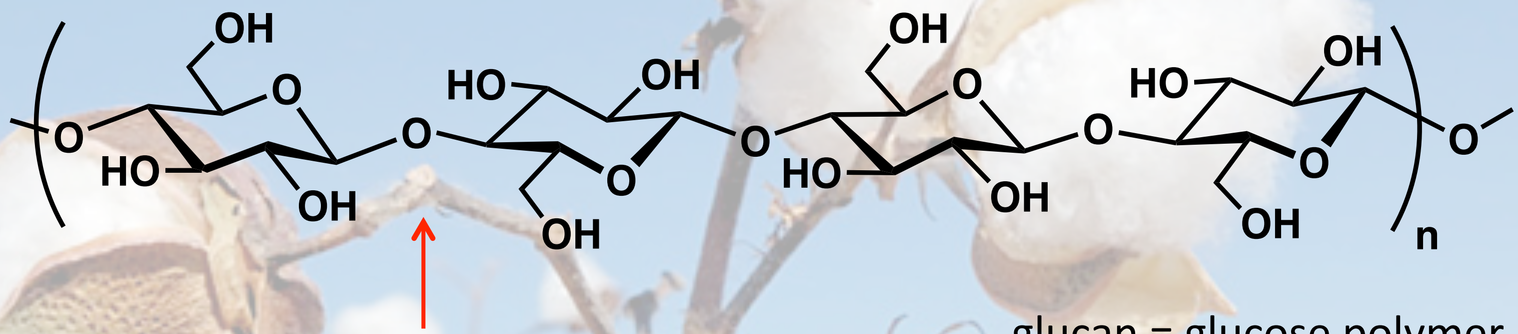
  cellulose, hemicelluloses, starch

- POLYCATIONIC
  chitin, chitosan

- POLYANIONIC

- glycosaminoglycans

Cellulose

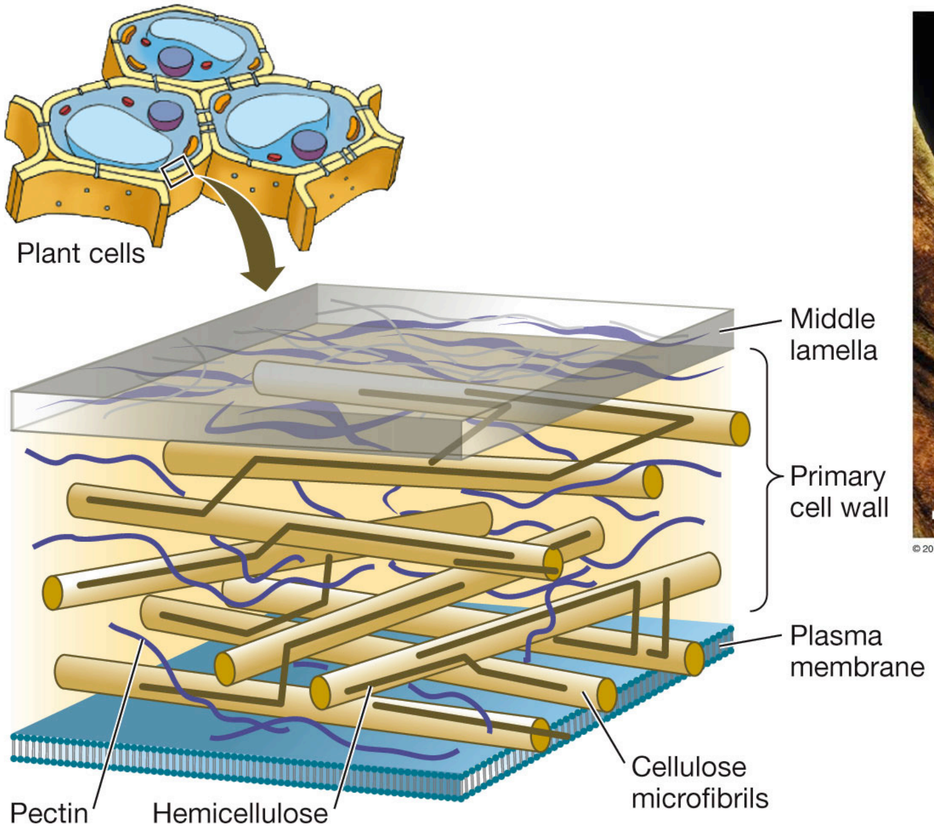


β-(1,4)-glycosidic bond

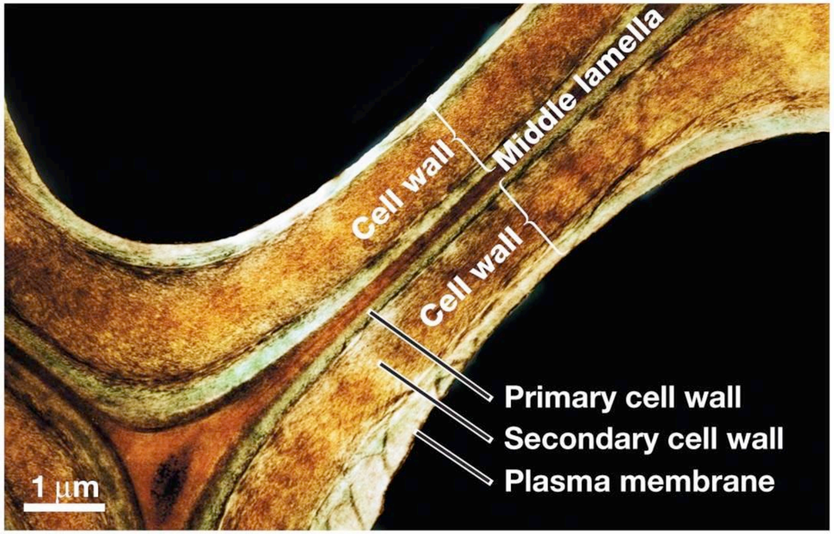
glucan = glucose polymer

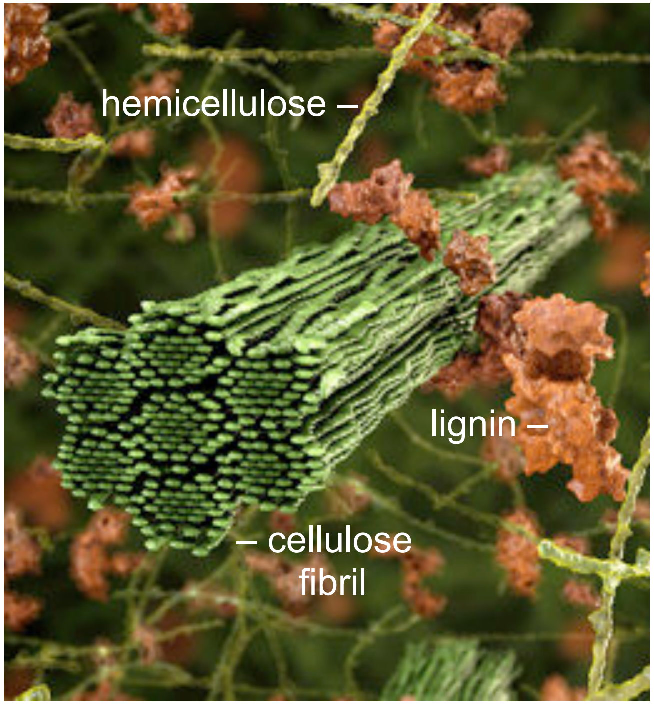
- Main component of plant cell wall
- Most abundant organic molecule on Earth (1.3x10^9 tons biosynthesized annually)
- One tree generates ~ 14g of cellulose/day
- 100-200 x 10^6 tons processed annually for industrial use (paper, textiles)
- Natural cellulose is never pure and is typically contaminated with hemicelluloses, pectin and lignin.
- Cotton fiber consists of 94% cellulose.

Structure of the plant cell wall



LIFE 9e, Figure 34.5

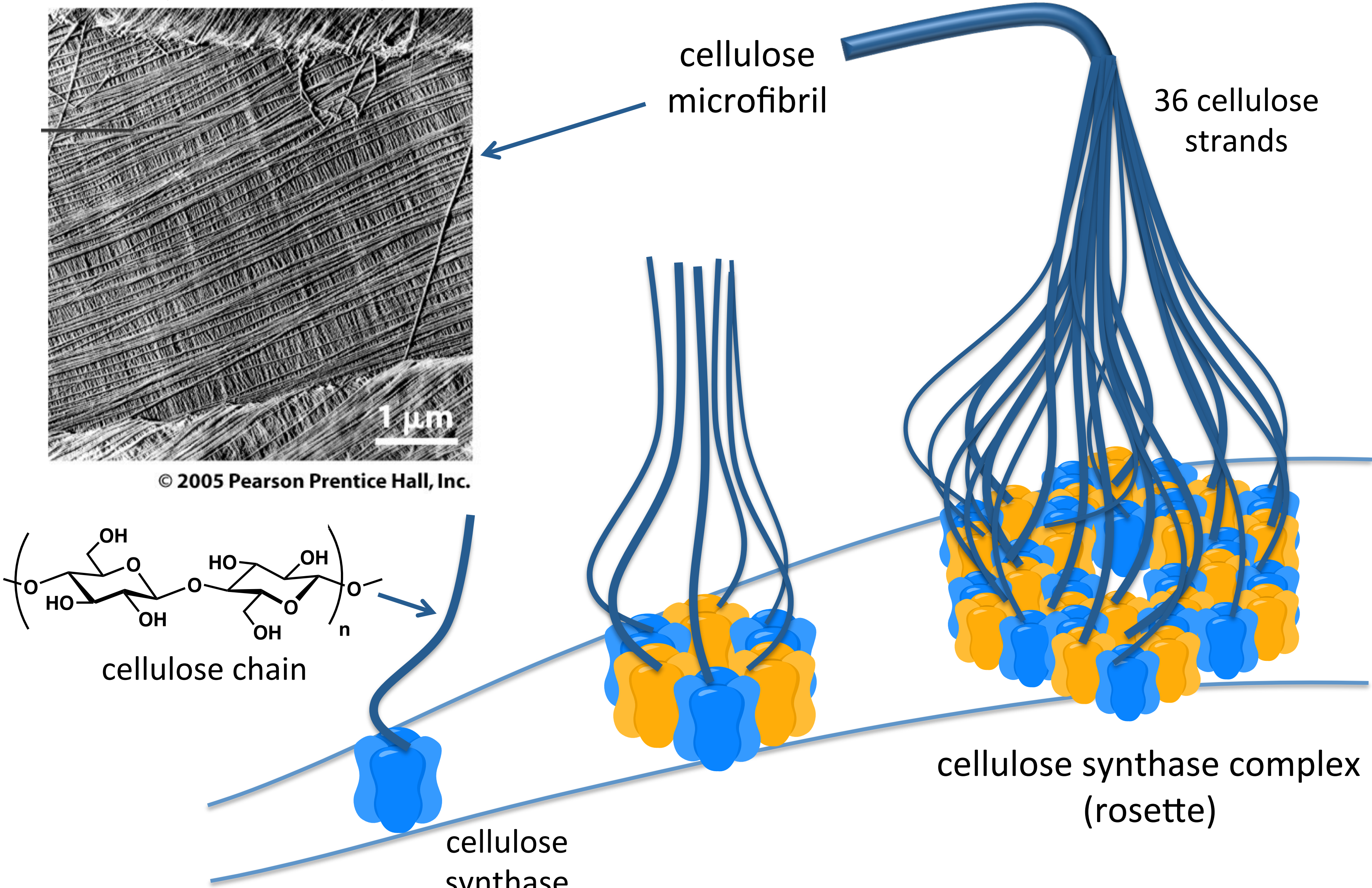




- Primary cell wall: ~ 8% cellulose the rest are hemicelluloses, lignin and proteins
- Secondary cell wall: ~ 95% cellulose

Cellulose biosynthesis

EM of a plant cell wall (top view)



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cellulose chain

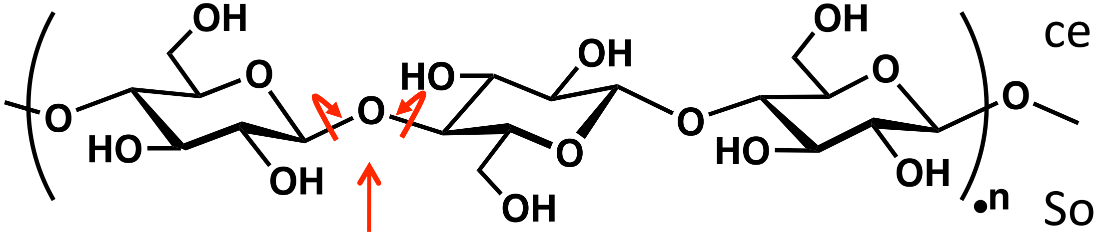
cellulose microfibril

36 cellulose strands

cellulose synthase

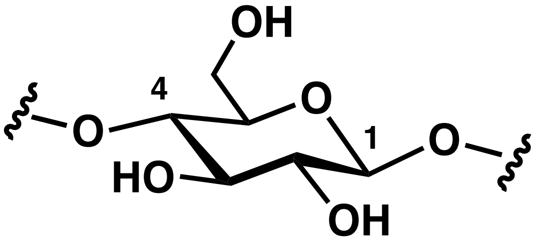
cellulose synthase complex (rosette)

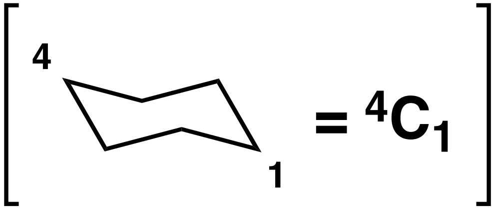
Molecular structure of cellulose



restricted rotation

- Degree of polymerization (DP or n) ~ 1,000-15,000 and depends on cellulose source (cotton: DP ~ 10-15k).
- Some degradation occurs during isolation, therefore, Mw of native cellulose can only be estimated.
- All substituents are in equatorial positions around a ring in a stable $^4C_1$ chair conformation:

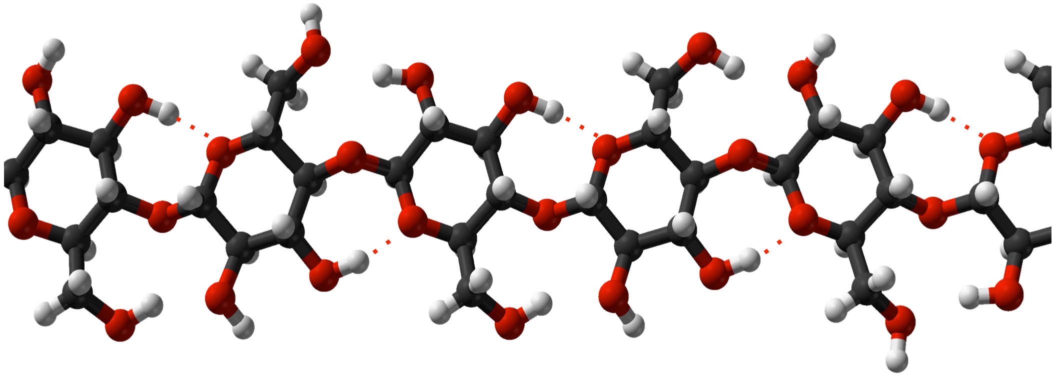


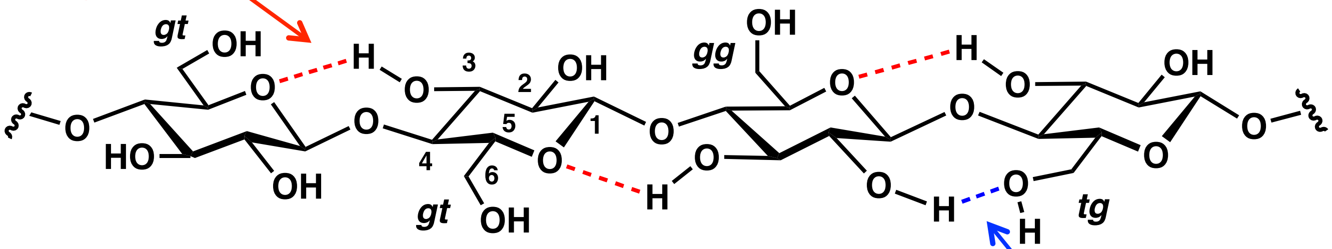


- Considerably rigid chain with restricted rotation around diequatorial β-(1,4) glycosidic bonds

Cellulose has an extended helical structure

- Single 2-fold helix
- 10.36 Å periodicity
- Stabilized through hydrogen bonding:

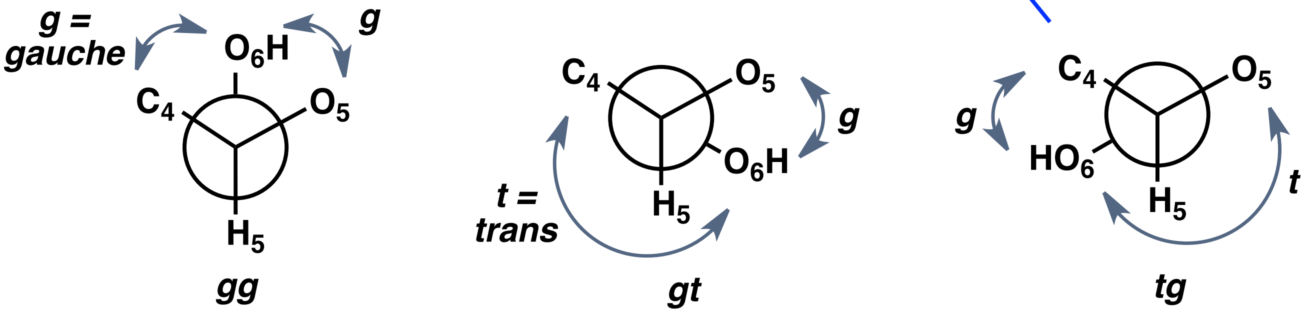




O_3H-O_5 H-bond
2.75 Å

O_2H-O_6 H-bond (2.87 Å)
in a *tg* conformation

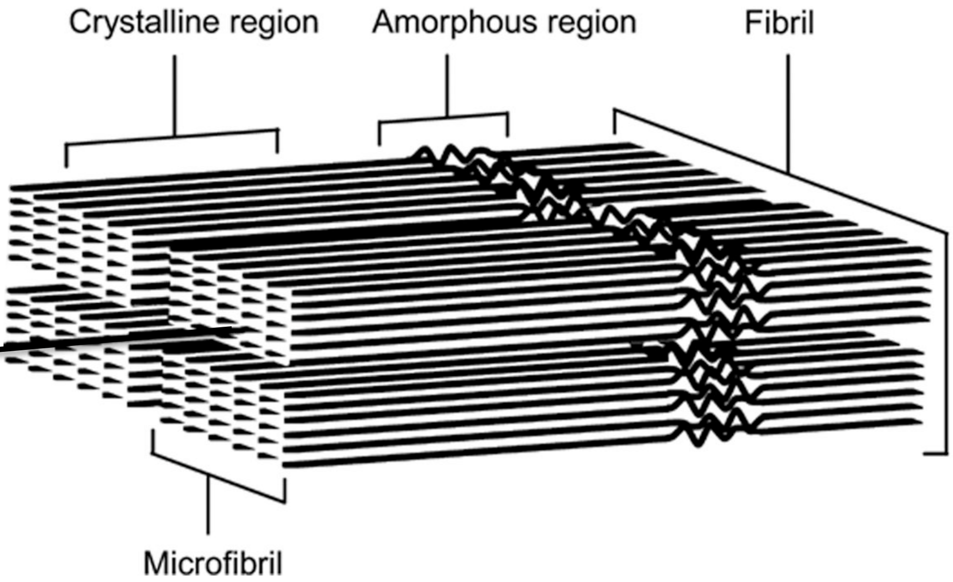
Three low energy conformations of C_6 hydroxyl group :



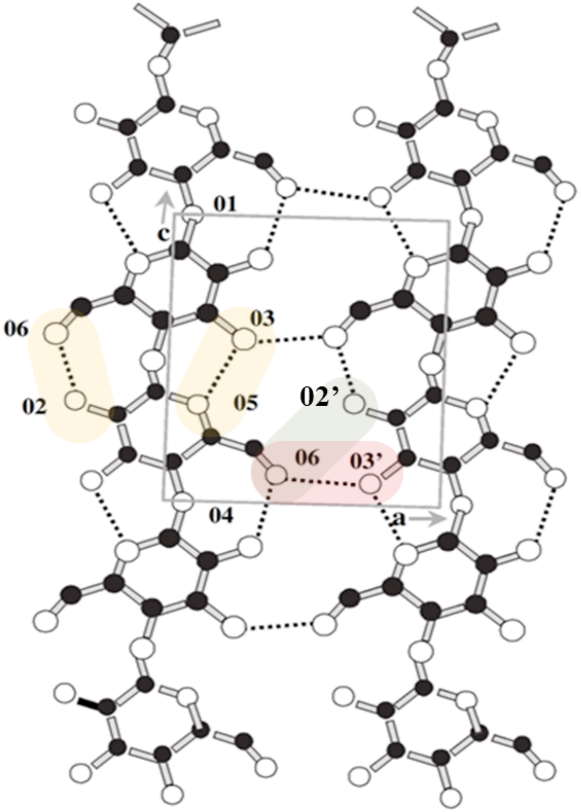
Newman projections down C5-C6 bond

Supramolecular structure of cellulose

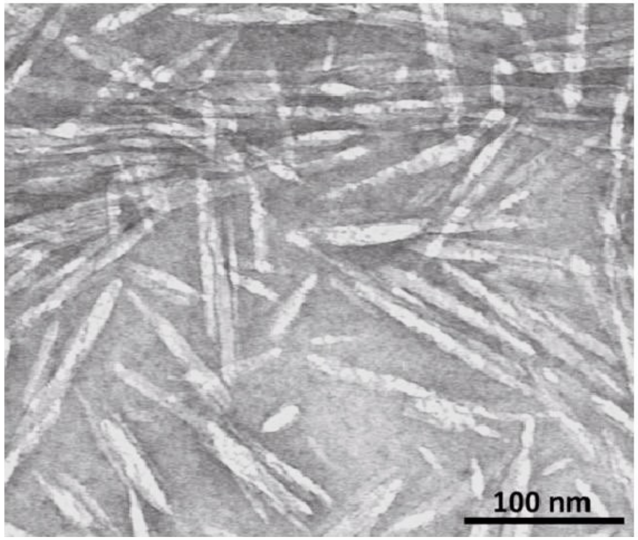
- Crystalline segments interrupted by amorphous regions
- Degree of native cellulose crystallinity ~ 60% (cellulose I)



cellulose helices form sheets stabilized by H-bonding:



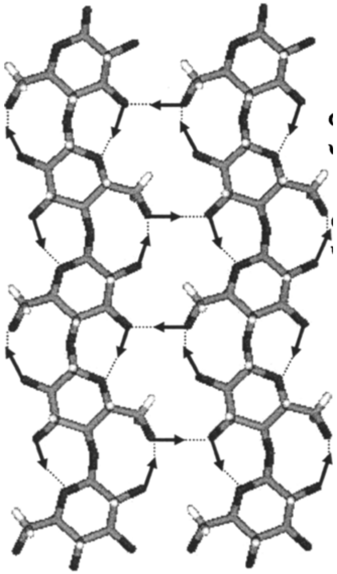
cellulose Iα/β

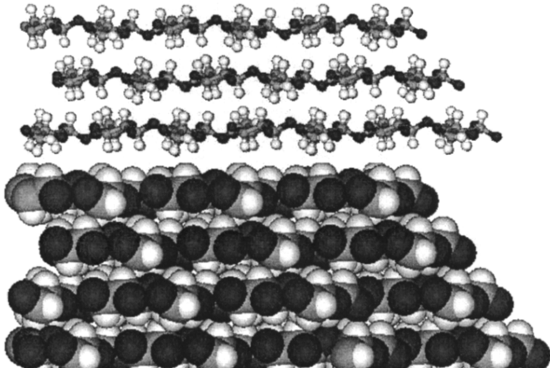


Allomorphs of natural cellulose I, α and β

Cellulose Iα:

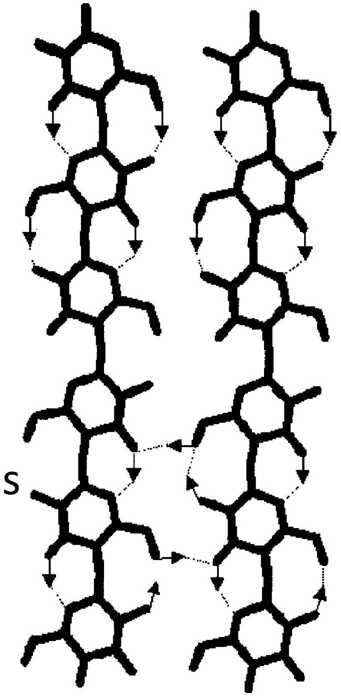
- monoclinic unit cell
- two parallel chains
- close packing
- 2-fold screw
- O_6H in tg
- intrachain O_3-O_5 H-bonds
- inter-chain H-bonding
- no inter-sheet H-bonds

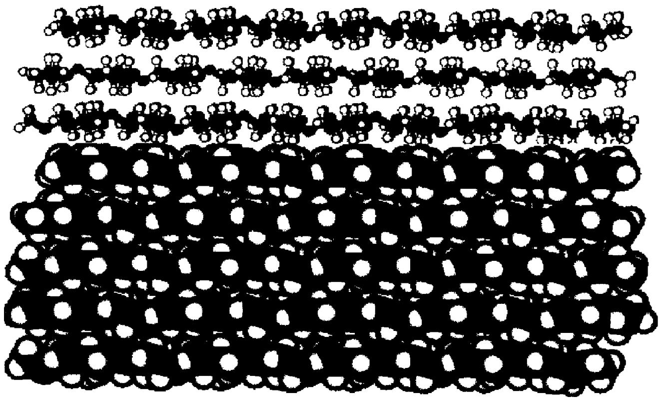




Cellulose Iβ:

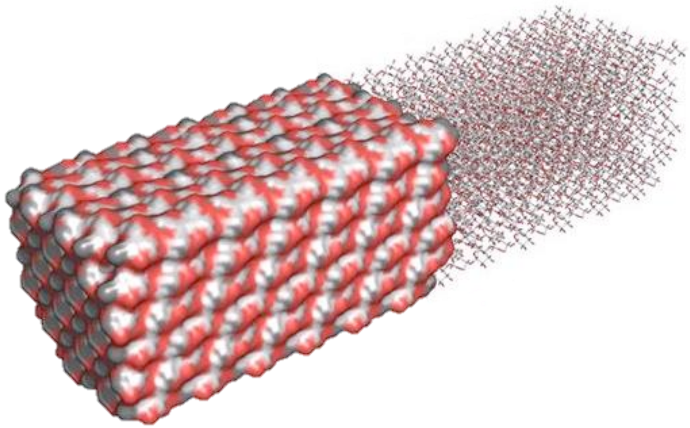
- triclinic unit cell
- two parallel chains
- different confromations
- 2-fold screw
- all O_6Hs in tg
- intrachain O_2-O_6 H-bonds
- inter-chain H-bonding
- no inter-sheet H-bonds

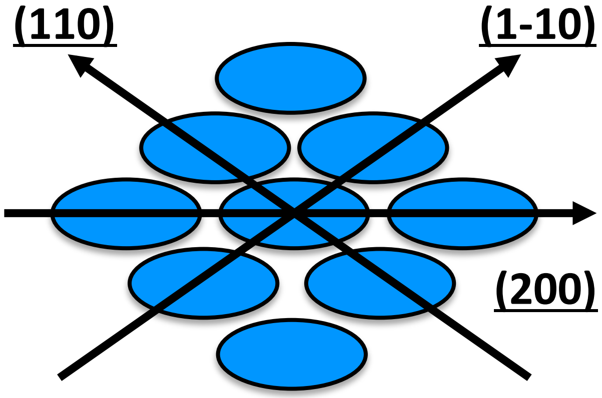


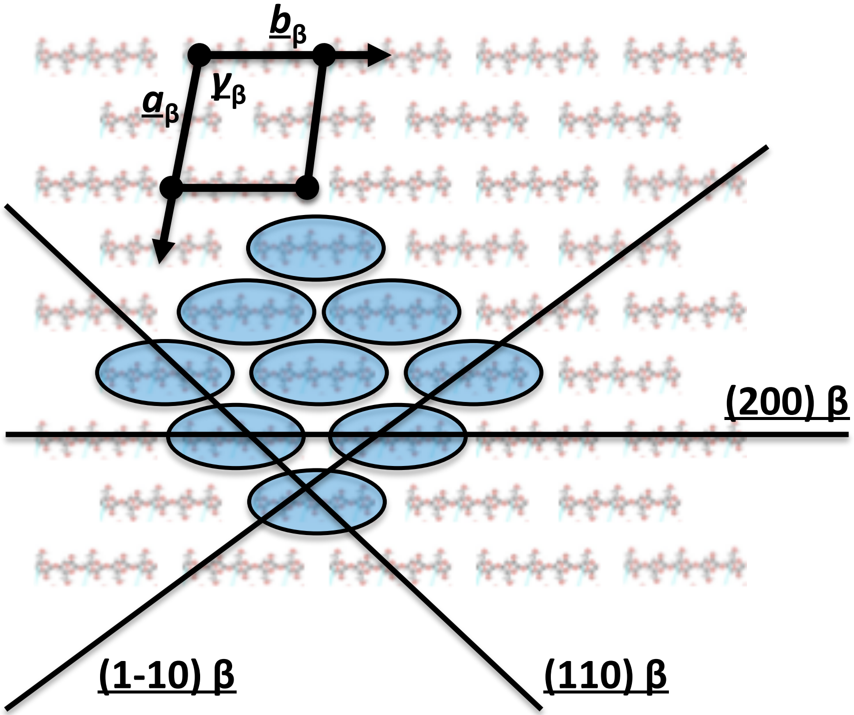


Nishiyama, Langan, Chanzy *JACS* 2002, 124, 9074–9082.

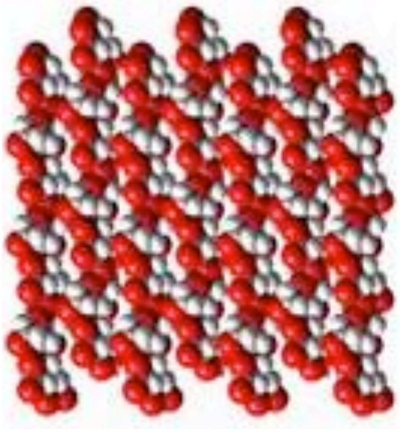
Polarity of cellulose crystallites (I_β)





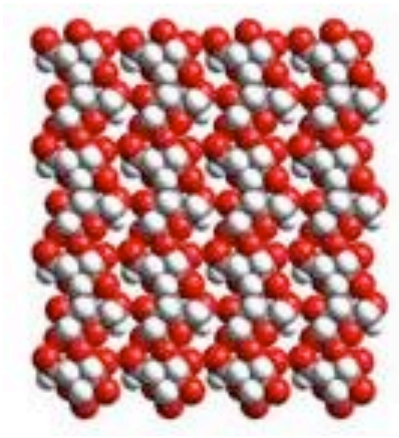


(110)



rough with exposed
OH groups
hydrophilic

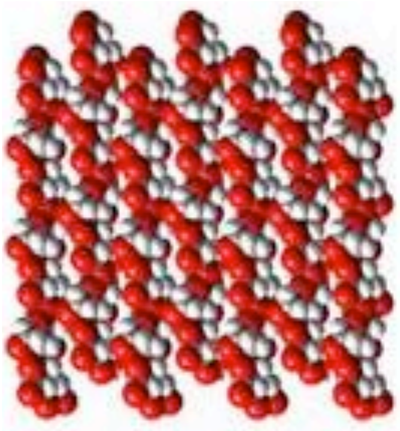
(200)



smooth with exposed
C-H groups
hydrophobic

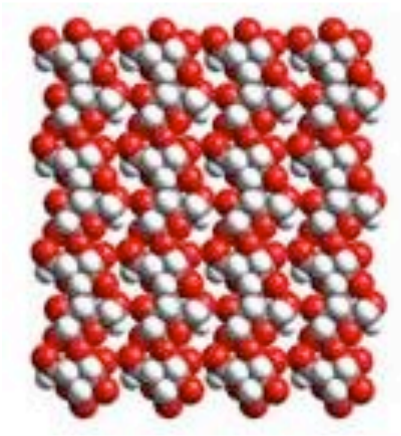
Supramolecular interactions with cellulose surfaces

(110)

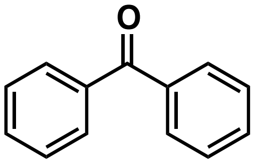


hydrophilic

(200)

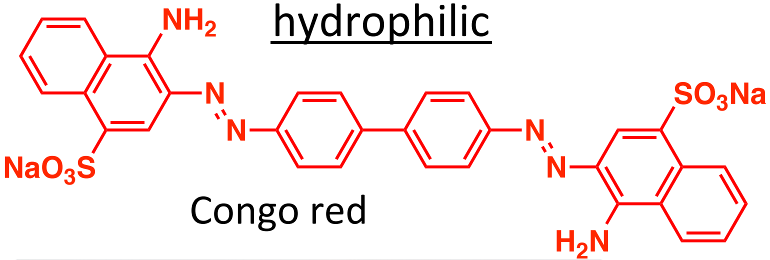


hydrophobic

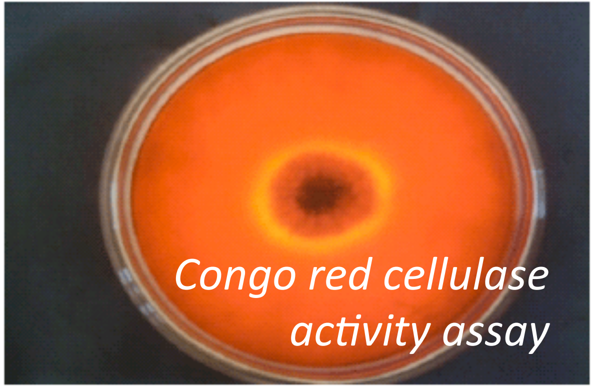


benzophenone

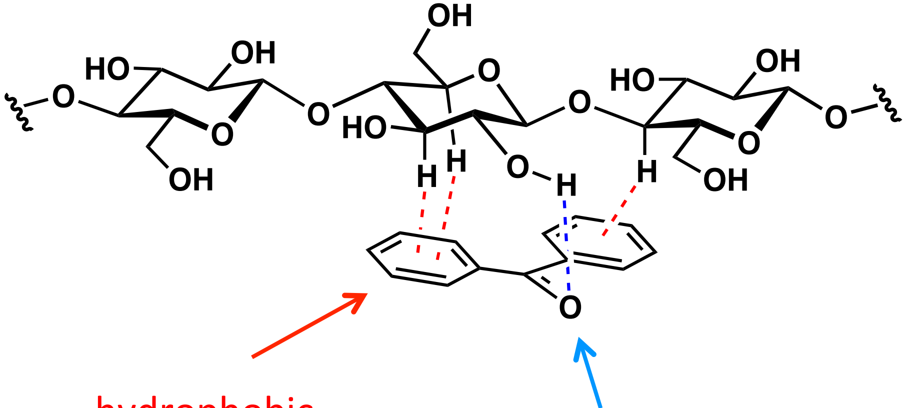
Monte Carlo and MD
Mazeau, K.; Vergelati, C.
Langmuir 2001, 18, 1919



Congo red



Congo red cellulase activity assay



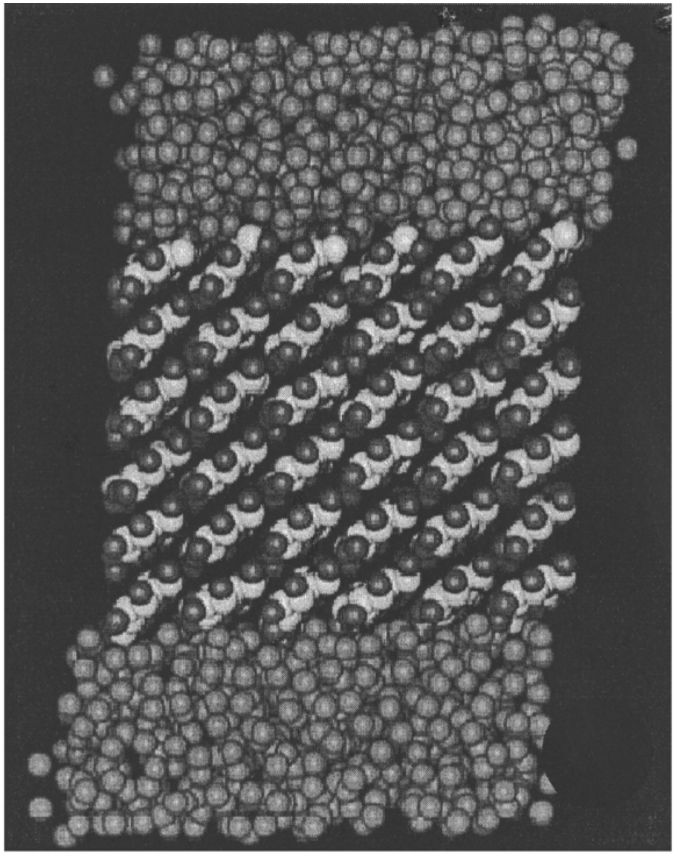
hydrophobic
C-H / π interactions

hydrogen bonding
between carbonyl
and OH grous

Molecular mechanics program, Assisted Model Building with Energy Refinement (AMBER) Woodcock et al *Biopolymers* 1995, 36, 201

Interactions of cellulose in biological settings

- Water
    - MD simulation, Gronengen Molecular Simulation System (GROMOS) force field.
    - Only the surface layer of the crystalline phase interacts in water.

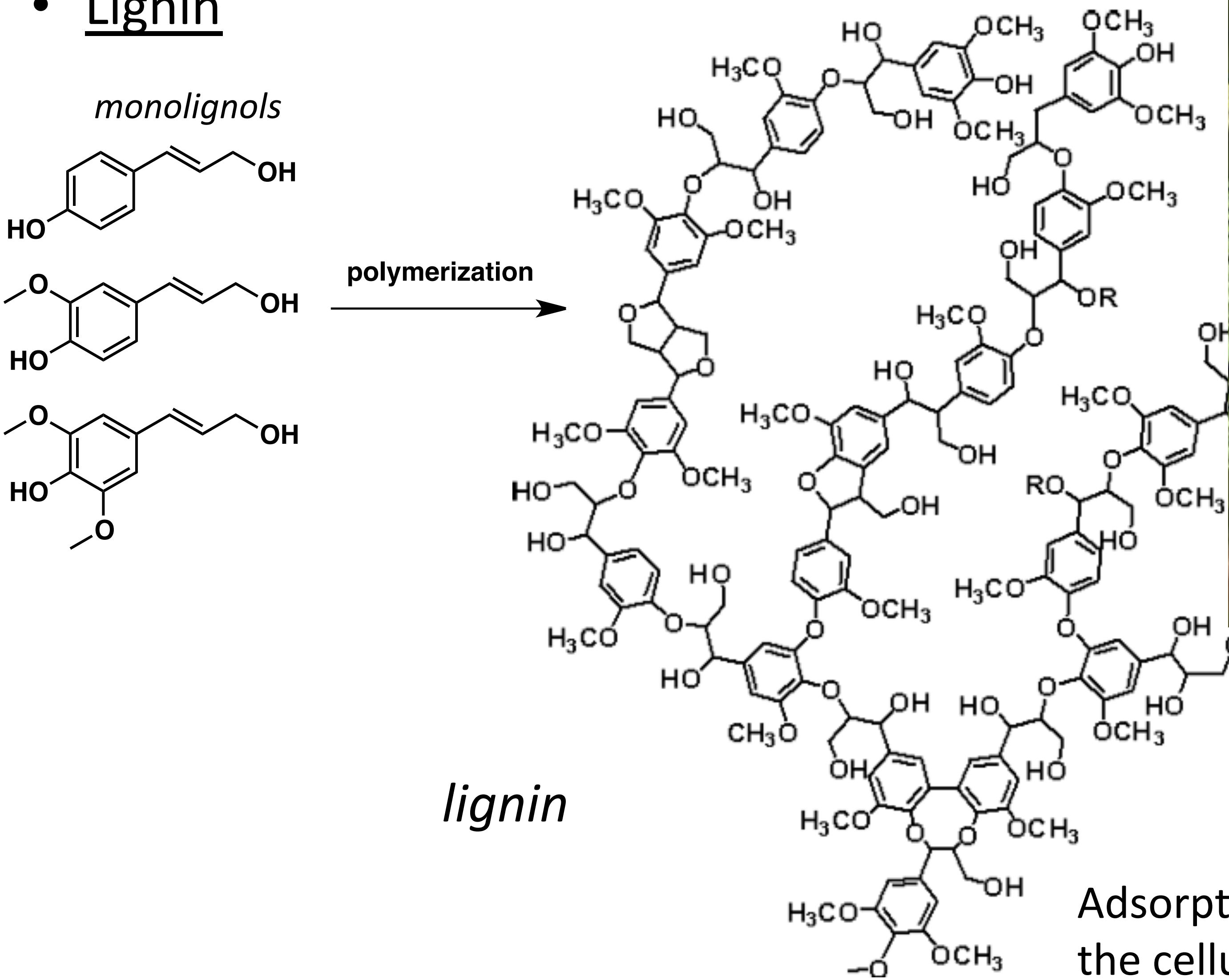


- Water becomes ordered at the crystal surface.
- Similarly the upper most cellulose molecules become disorganized due to loss of intra-chain hydrogen bonding.

Heiner and Teleman *Langmuir* 1997, 13, 511

Interactions of cellulose in biological settings

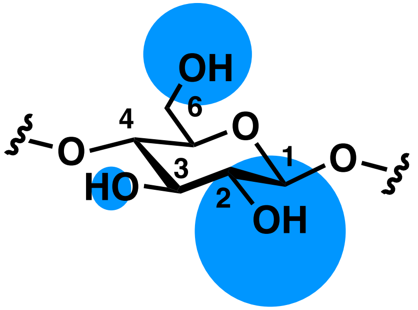
- Lignin



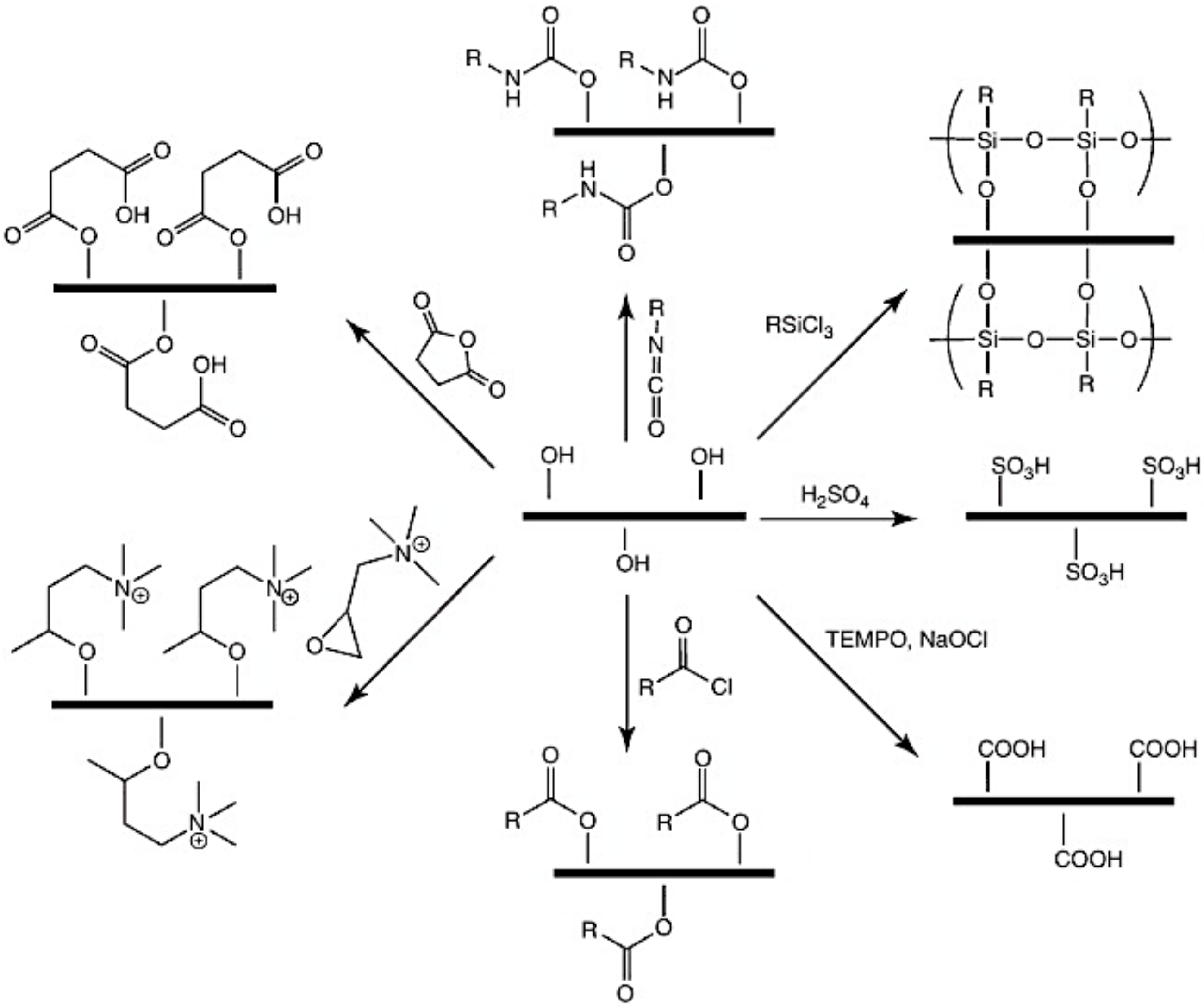
Adsorption of monolignols to the cellulose fiber can catalyze polymerization to lignin.

Houtman, C.; Atalla, R.J. *Plant Physiol*. 1995, 107, 977

Chemical modification of cellulose



● = relative reactivity

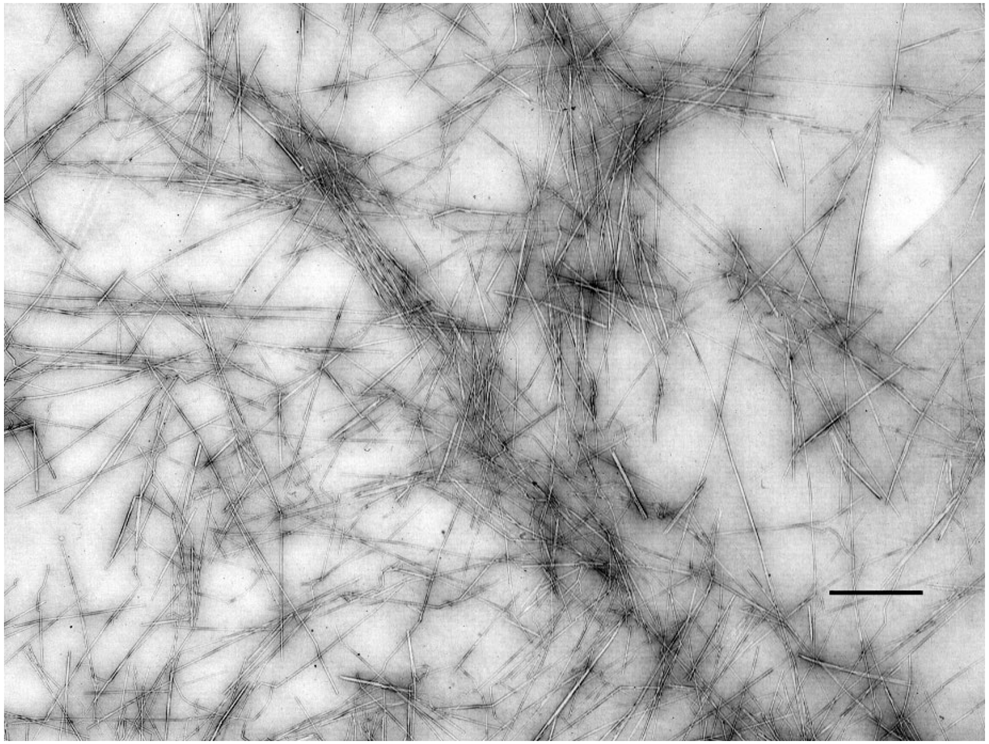


Hydroxyl groups involved in hydrogen binding exhibit lower propensity for chemical modification

Cellulosic nanomaterials: tunicate whiskers

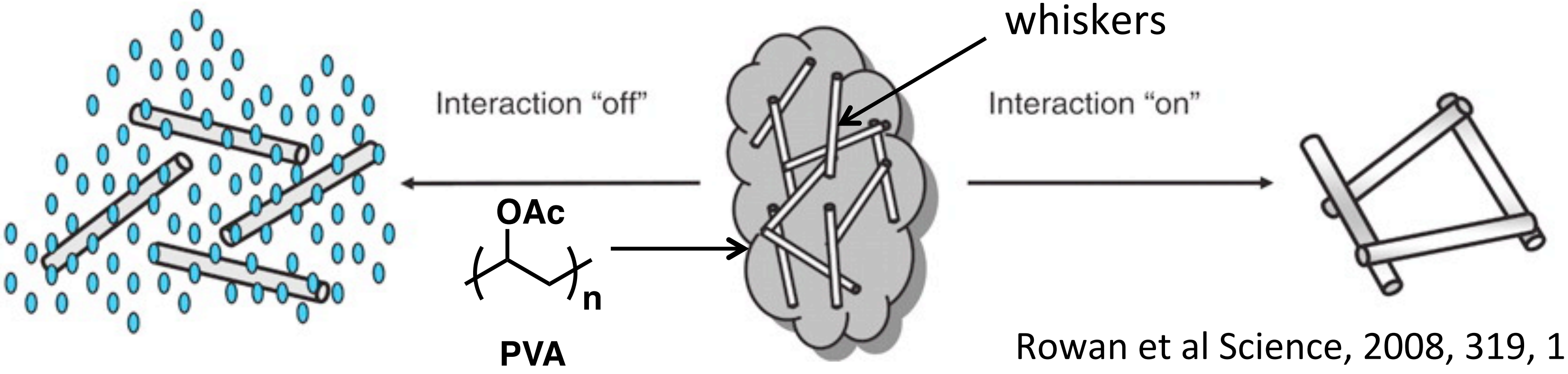
Tunicate

high aspect ratio ~ 26 nm x 2 µm



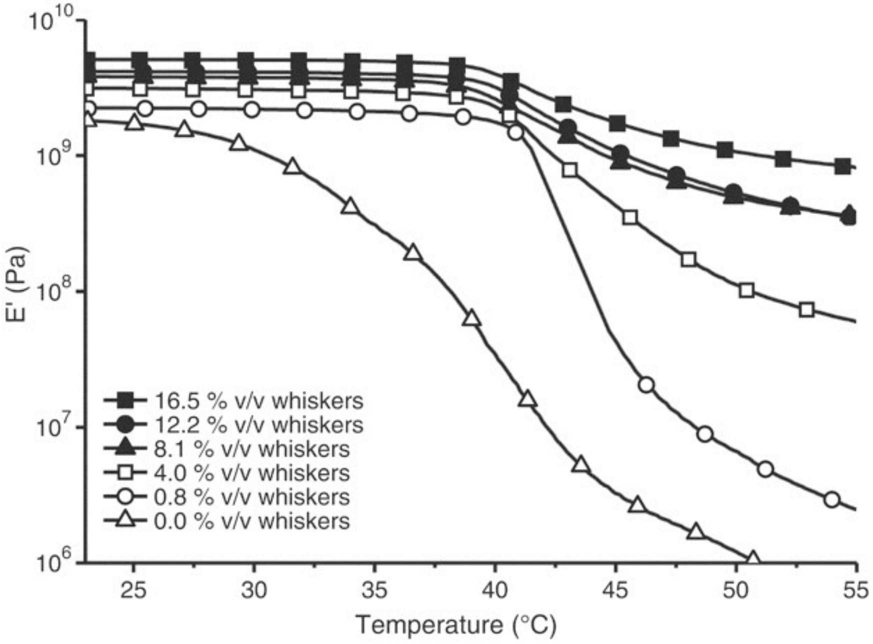
crystallinity of nanowhiskers decreases with diameter due to increase in the proportion of disordered surface chains

Mechanically adaptive cellulose materials

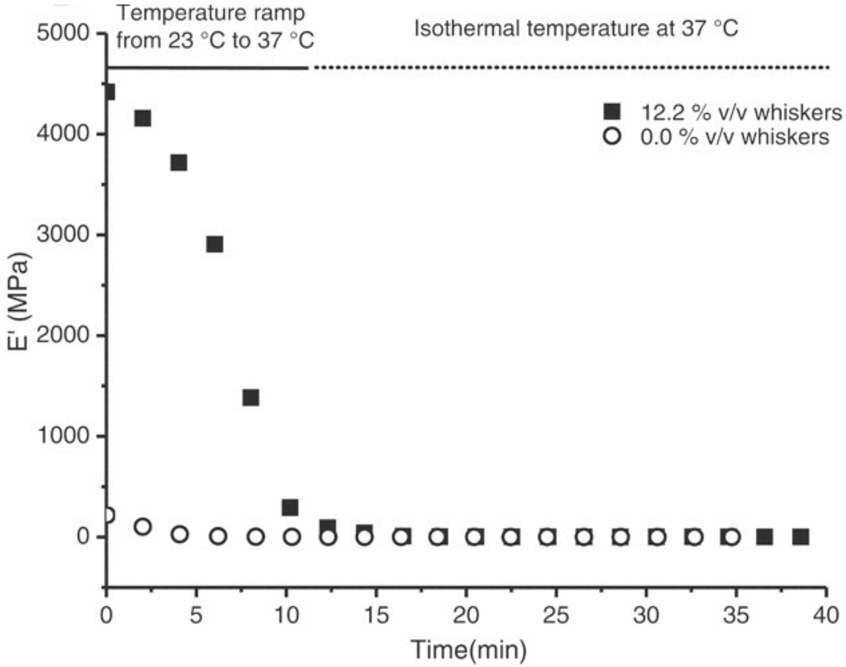


Rowan et al Science, 2008, 319, 1370

Tensile storage modulus of dry PVA/ whisker film as a function of temperature:



Tensile storage modulus of PVA/whisker film in cerebrospinal fluid at 37°C:



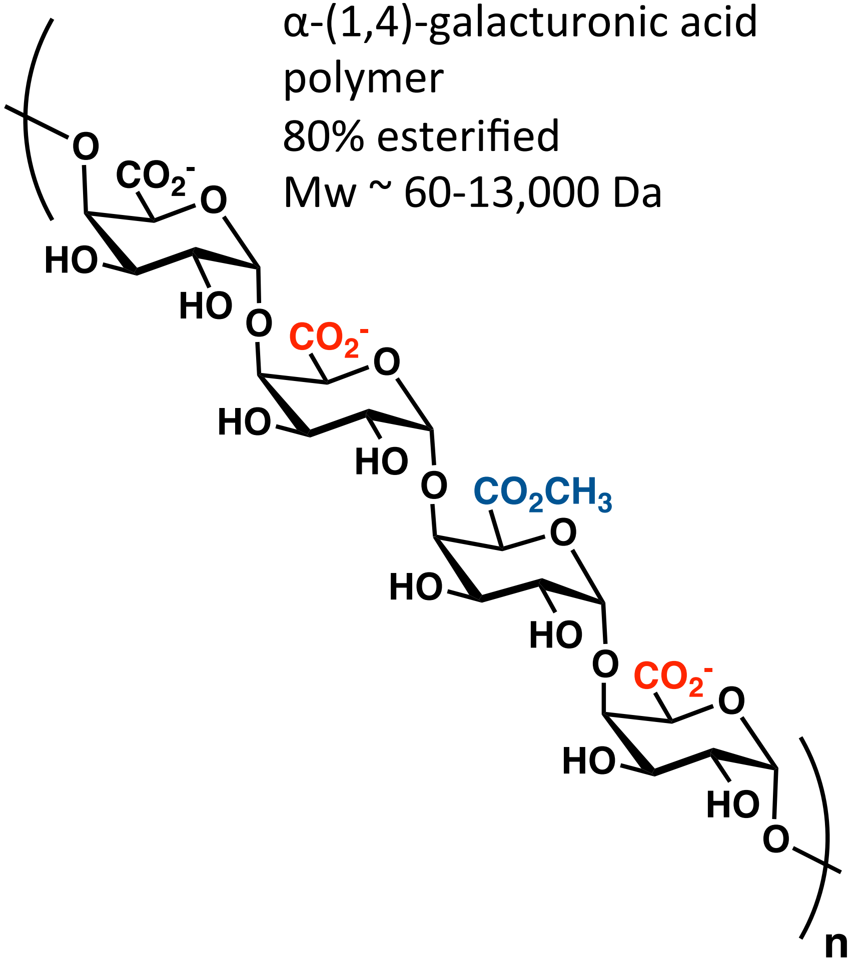
Hemicelluloses of terrestrial plants

Pectin is a major component of the middle lamella and the primary cell wall of terrestrial plants. It helps bind cells together and allows for growth.

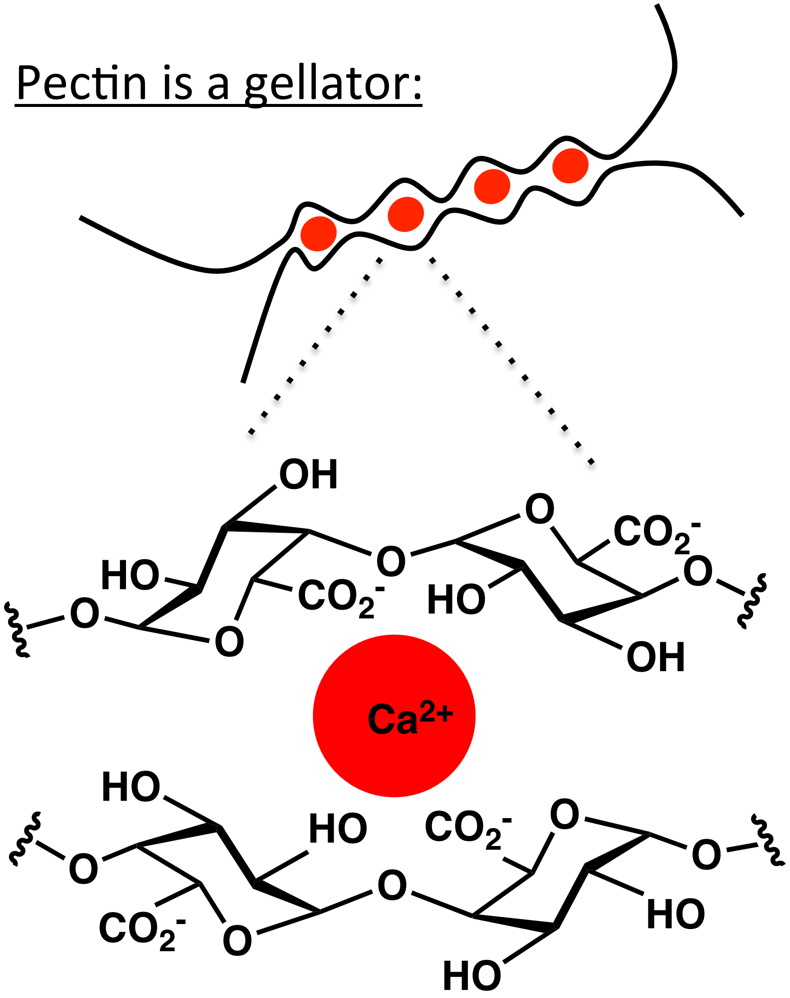
α-(1,4)-galacturonic acid polymer

80% esterified

Mw ~ 60-13,000 Da

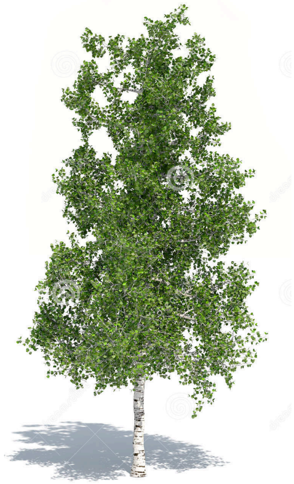


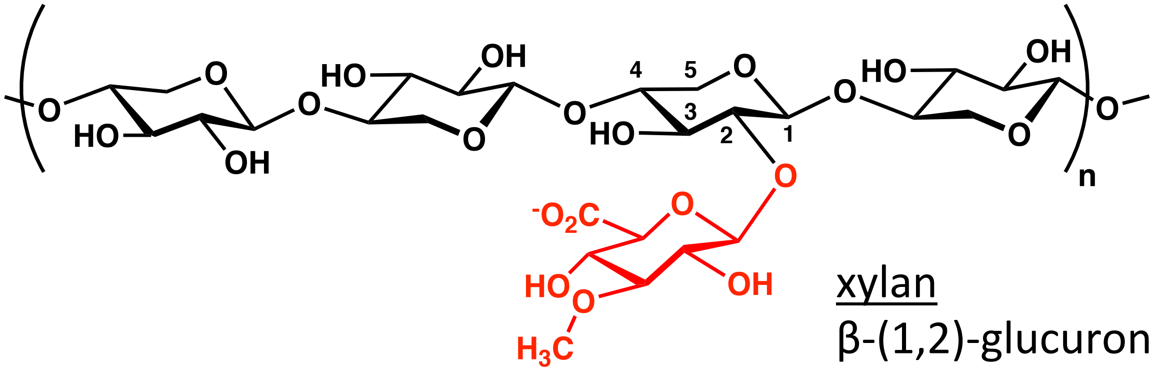
Pectin is a gellator:



Hemicelluloses of terrestrial plants

Hardwood: e.g., birch, alder, balsa, hickory, mahogany, maple, oak, teak, or walnut.





xylan

β-(1,2)-glucuronyl-β-(1,4)-xylose

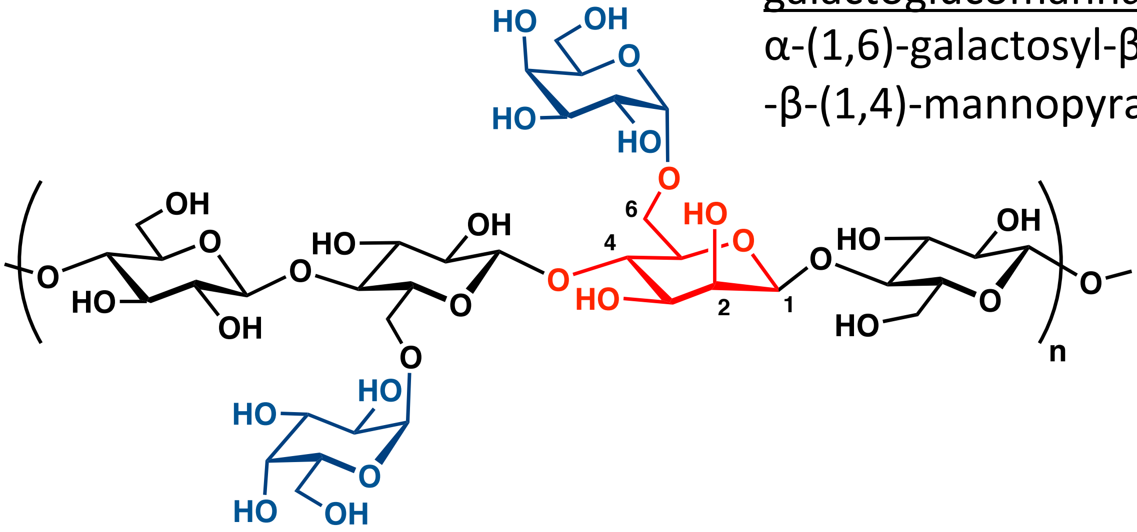
GlcA every 10th residue

Softwood: e.g., cedar, fir, juniper, pine, redwood, spruce, or yew.

galactoglucomannan

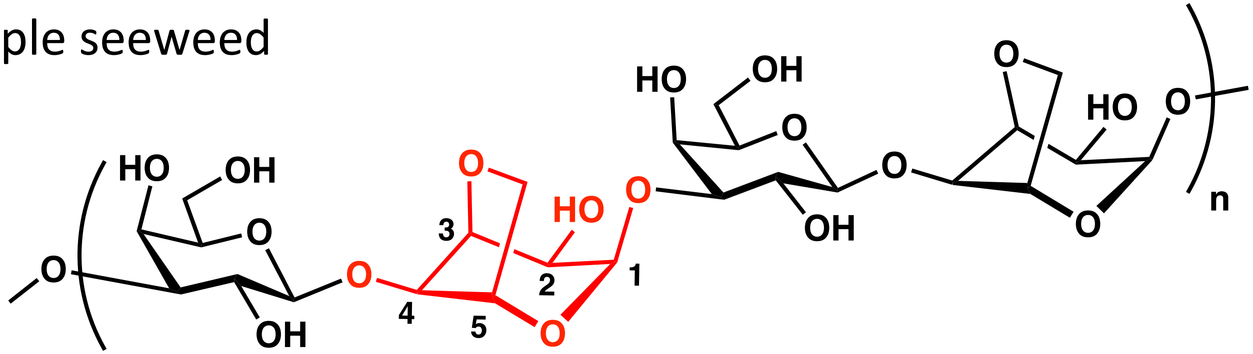
α-(1,6)-galactosyl-β-(1,4)-glucosyl-

-β-(1,4)-mannopyranoside

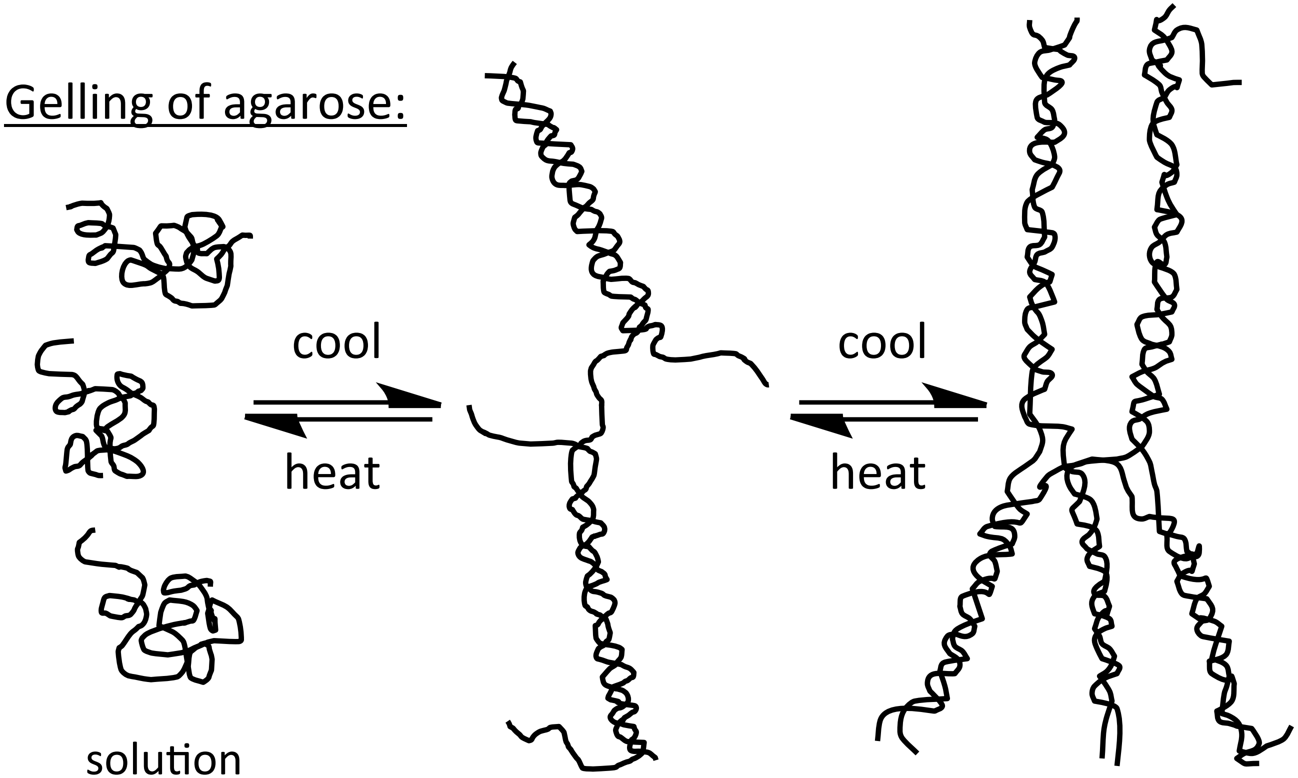


Hemicelluloses of marine algae

Agarose (Agar): Red-purple seeweed



Gelling of agarose:



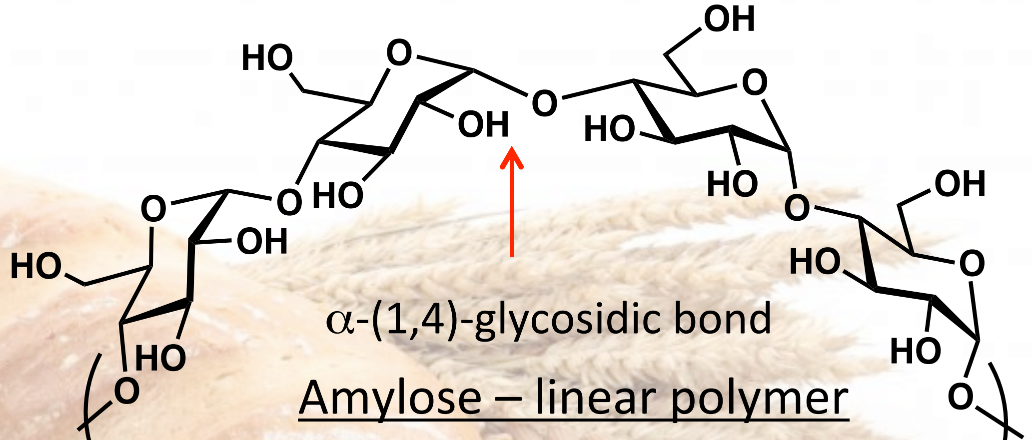
solution

gel I

gel II

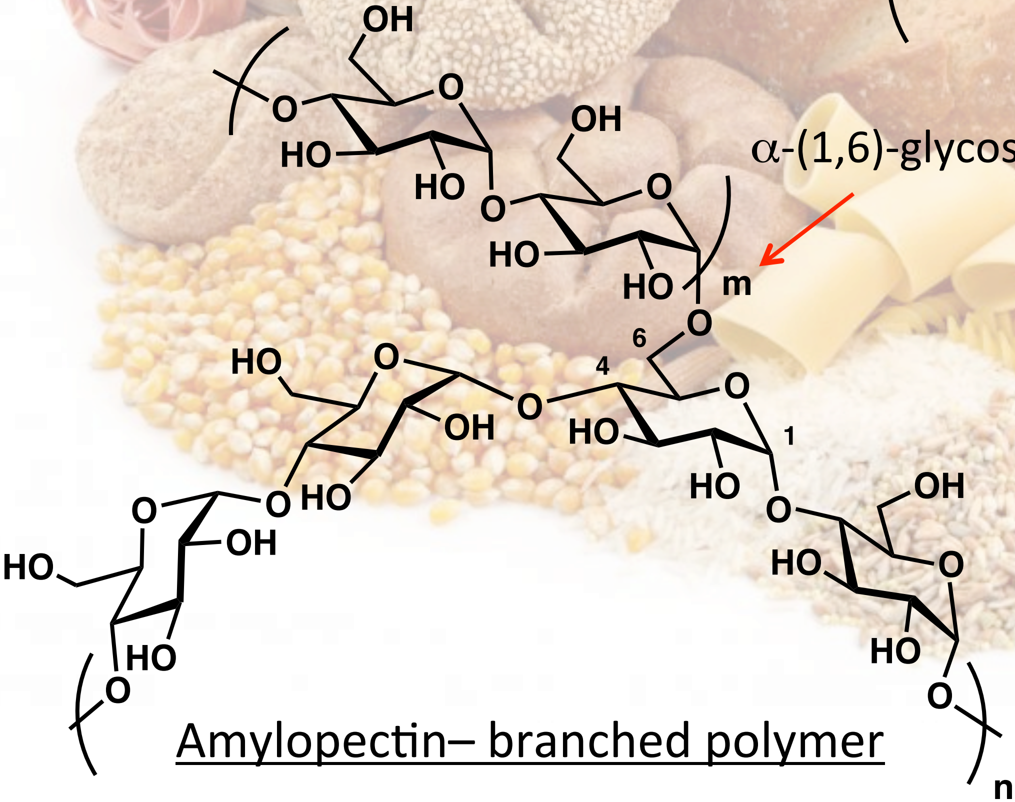
Starch

- Main component of human diet
- 2/3 of carbohydrate calories come from starch
- Slow breakdown



α-(1,4)-glycosidic bond

Amylose – linear polymer



α-(1,6)-glycosidic branch

Amylopectin– branched polymer

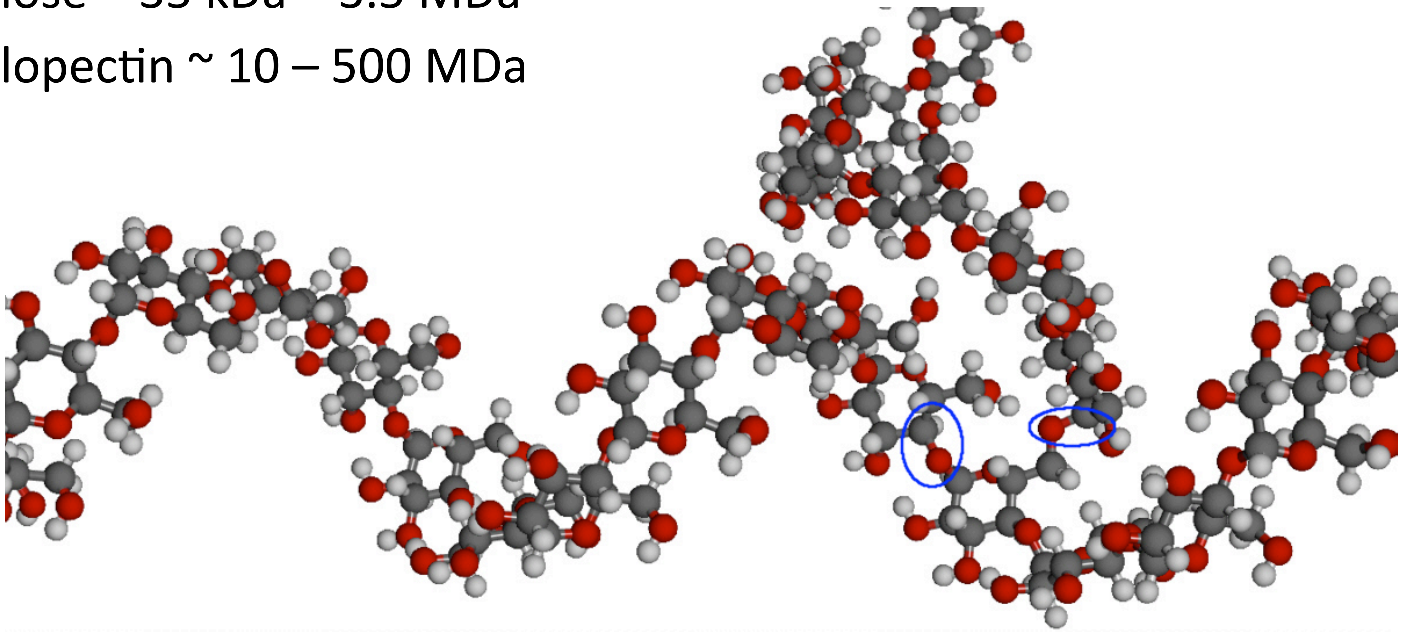
- Amylose/Amylopectin ratio (~ 1:3) is characteristic for different grains:

	% amylose
Potato	**18-23**
Rice	**16-30**
Rye	**24-26**

Amylose and amylopectin form helical structures

Amylose ~ 35 kDa – 3.5 MDa

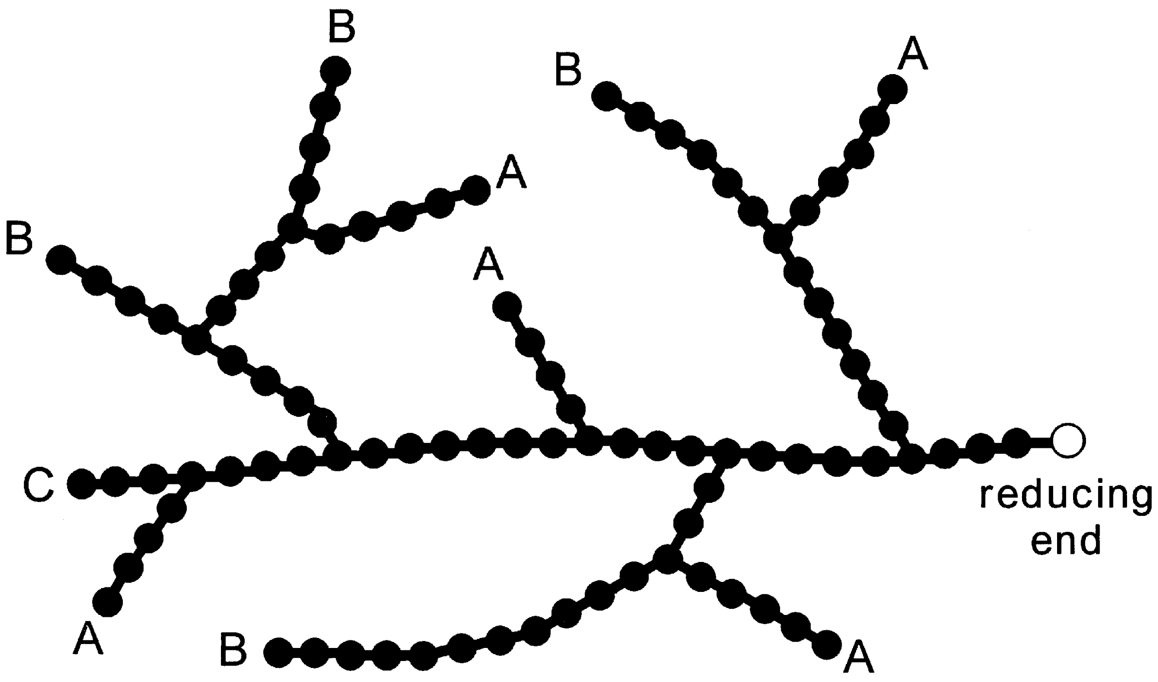
Amylopectin ~ 10 – 500 MDa



- amylose helices contain six α-Glc units per unit cell (helical turn) and three to four molecules of water of hydration
- left-handed helices being slightly more energetically favorable

Prof. Robert J. Lancashire, The Department of Chemistry, University of the West Indies,

Amylopectin structure



Chains in amylopectin:

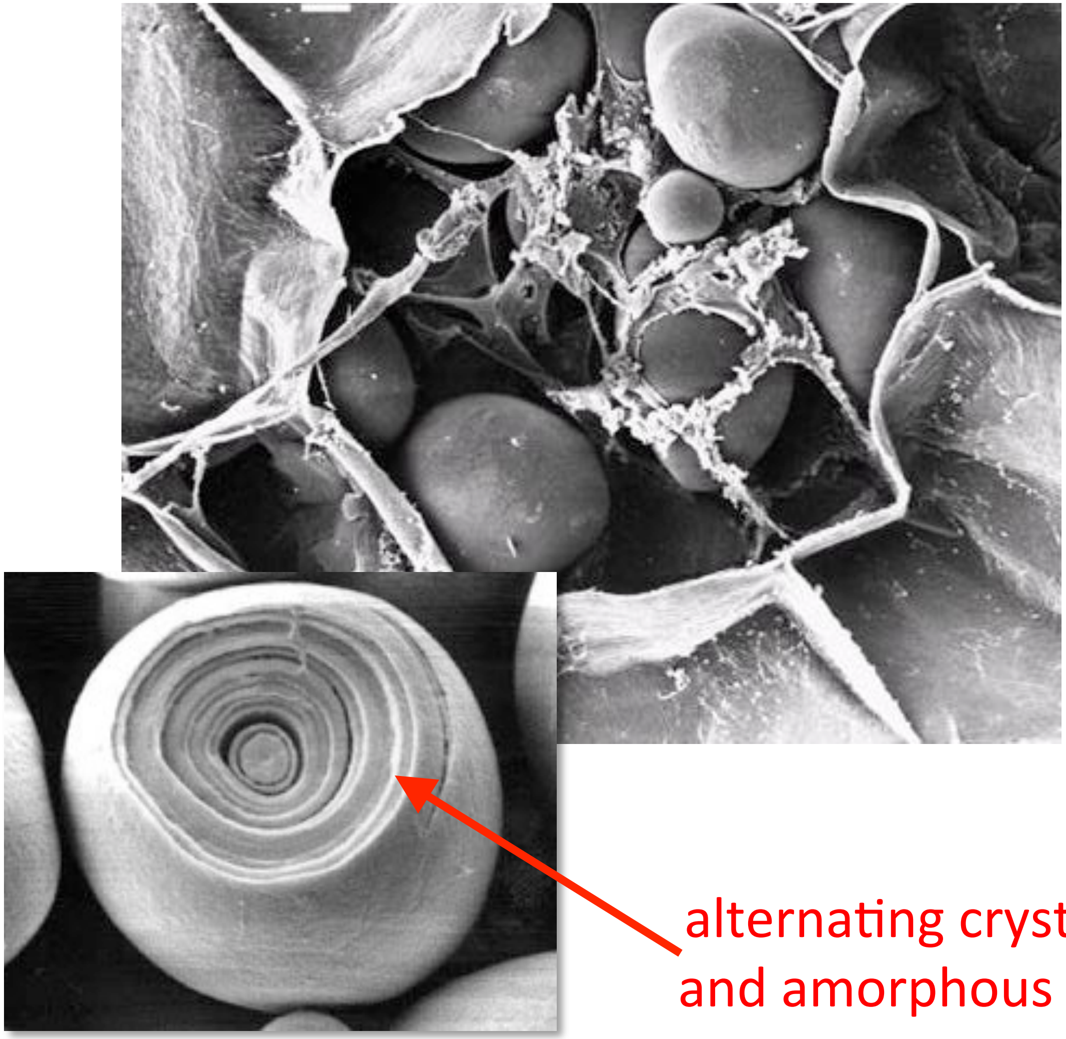
A = no branches and linked via reducing end

B = contains branches but no reducing end

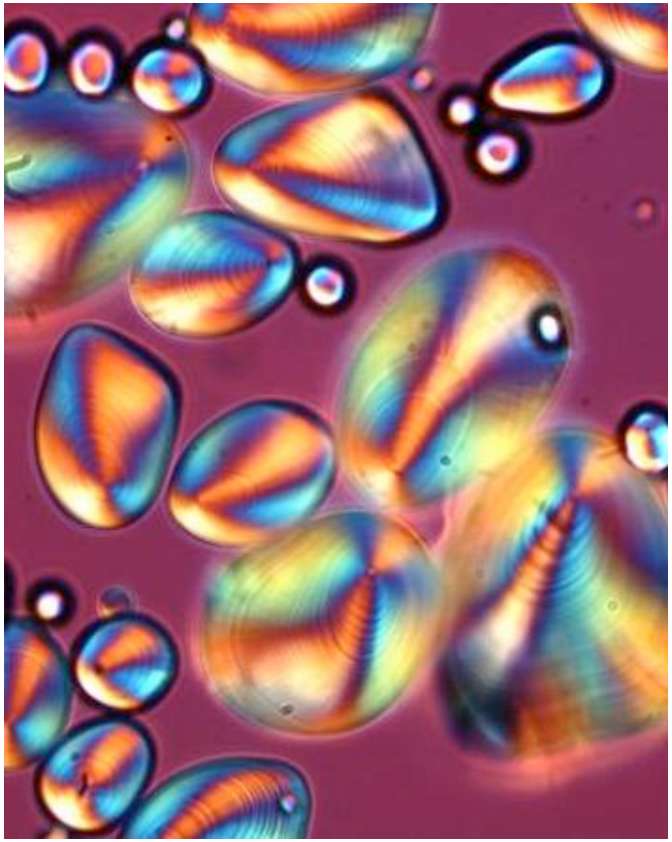
C = contains reducing end

Starch granule

Sizes and shapes of granule range from < 1 µm to > 100 µm and depend on grain.

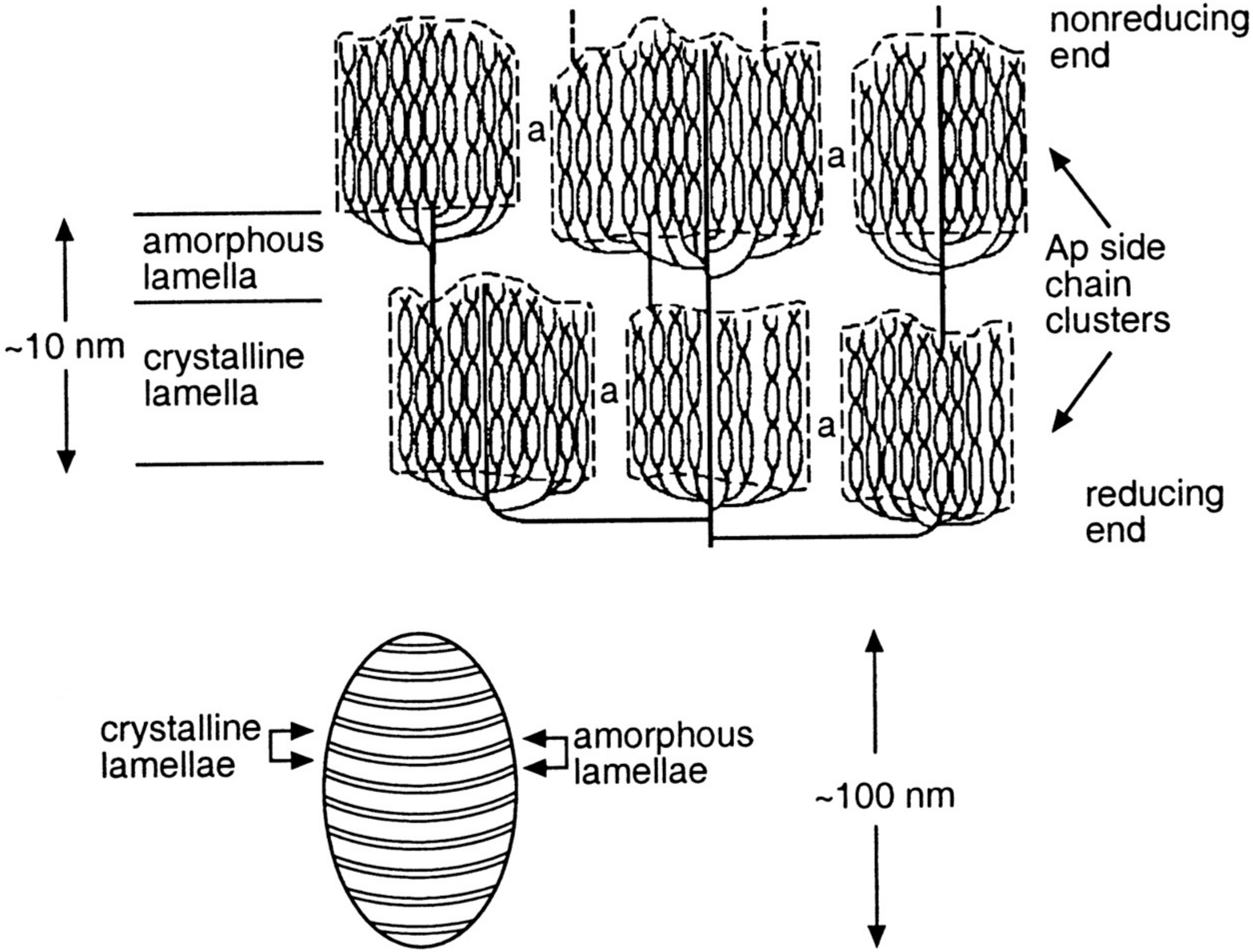


alternating crystalline and amorphous layers

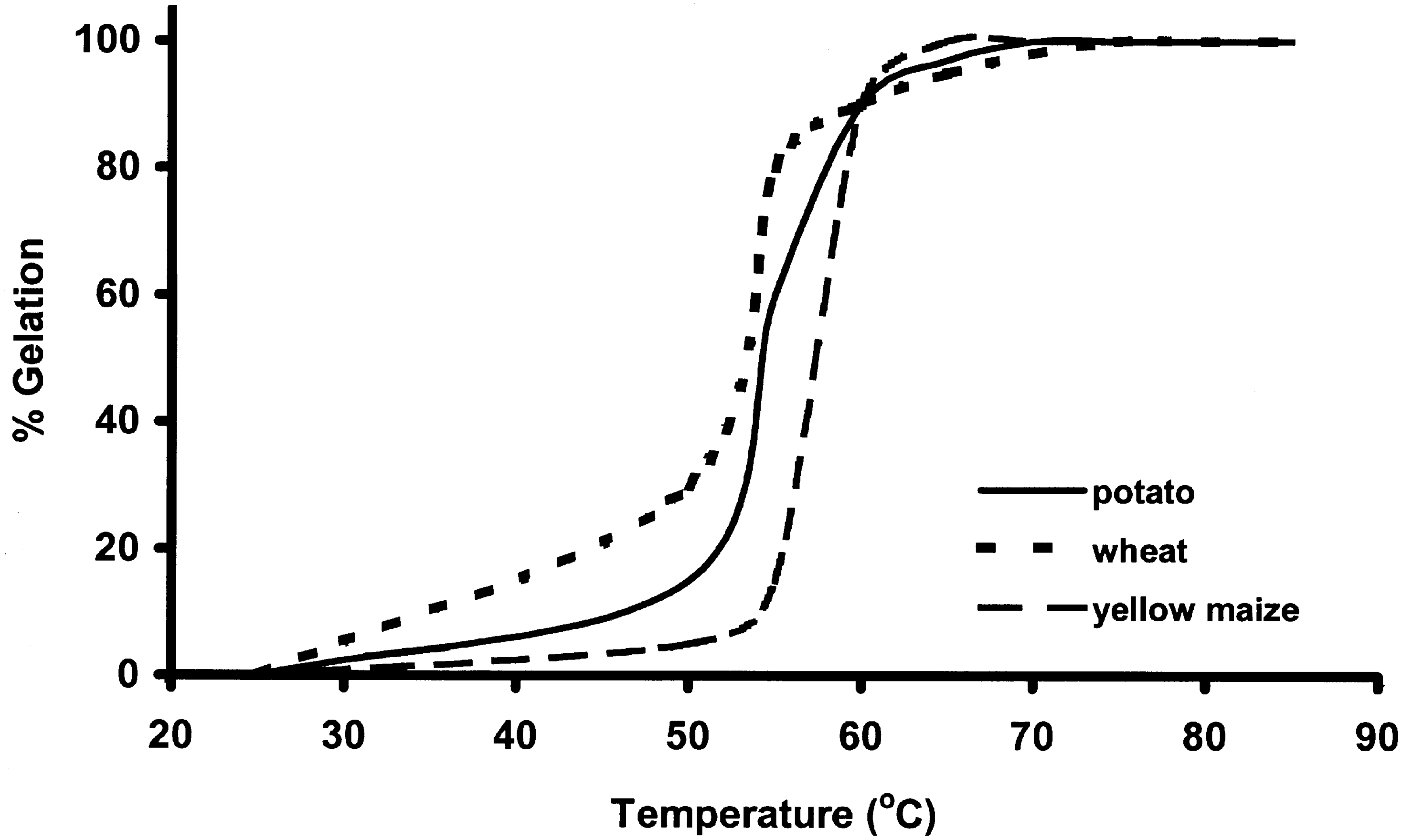


positive birefringence due to a radial organization of the crystallites

Starch granule and supramolecular structure



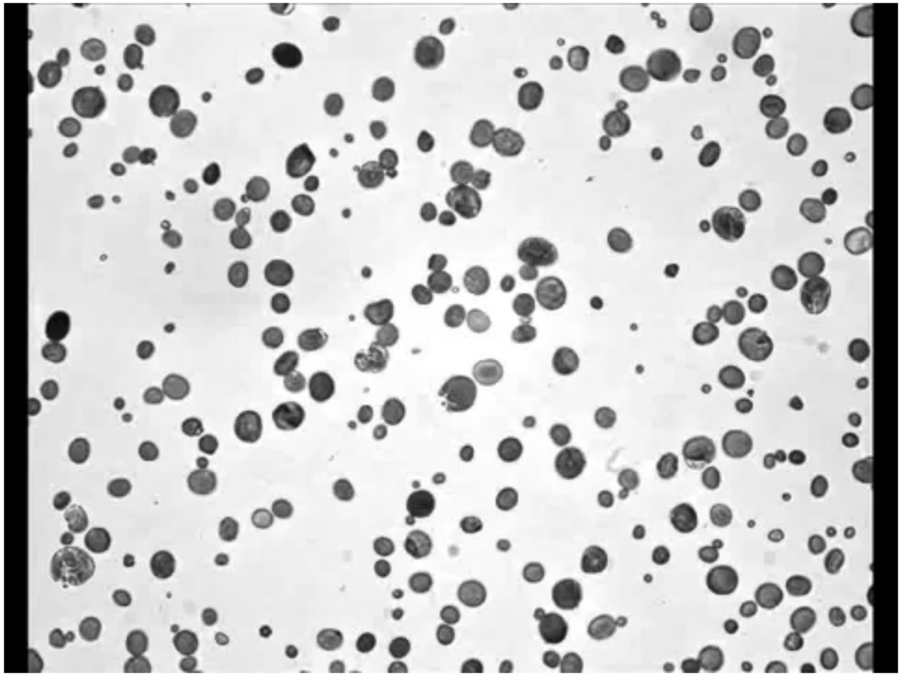
Gelatinization of starch of different botanical origin

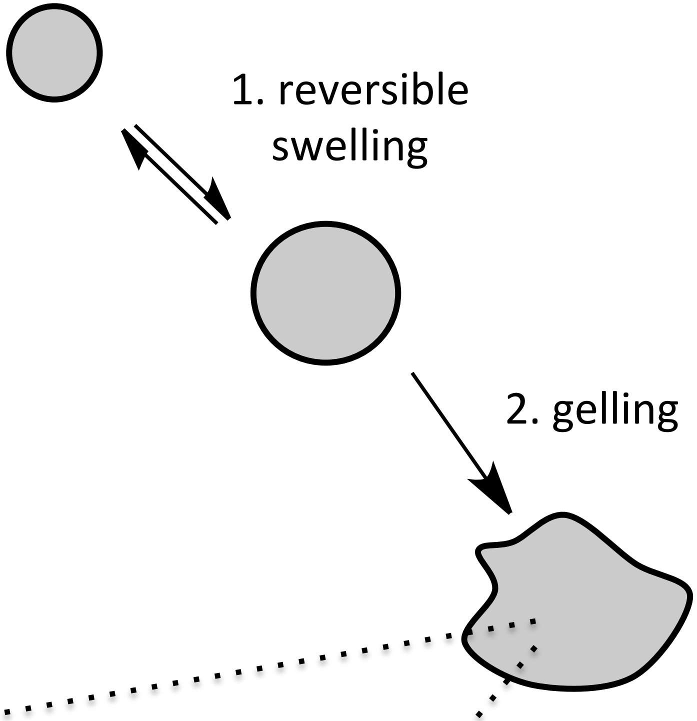


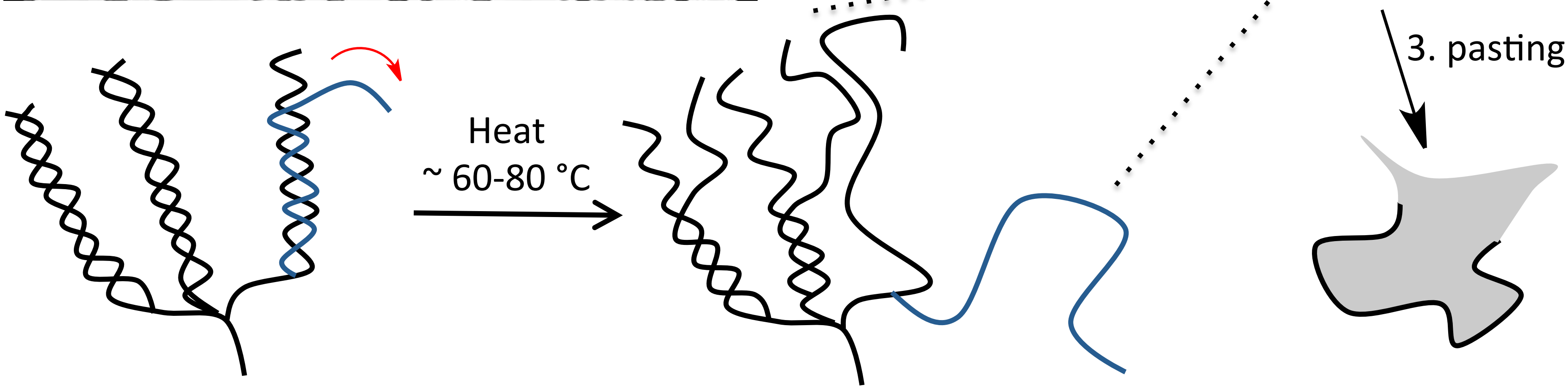
Charles J. Knill and John F. Kennedy

Gelatinization of starch

Heat Gelatinization of Wheat Starch:

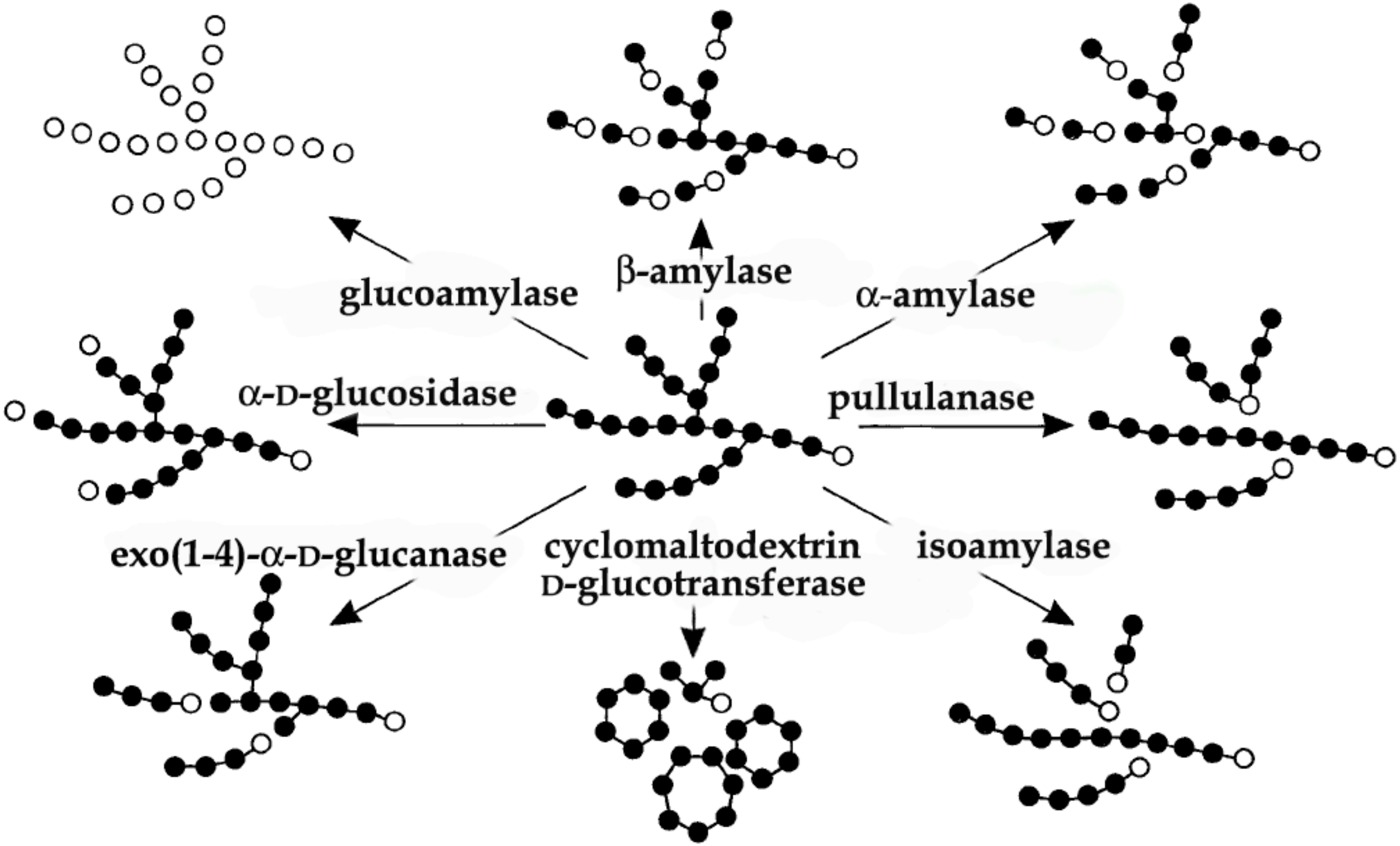






(GuelphCerealLab, https://youtu.be/L6vYxYE1jOg)

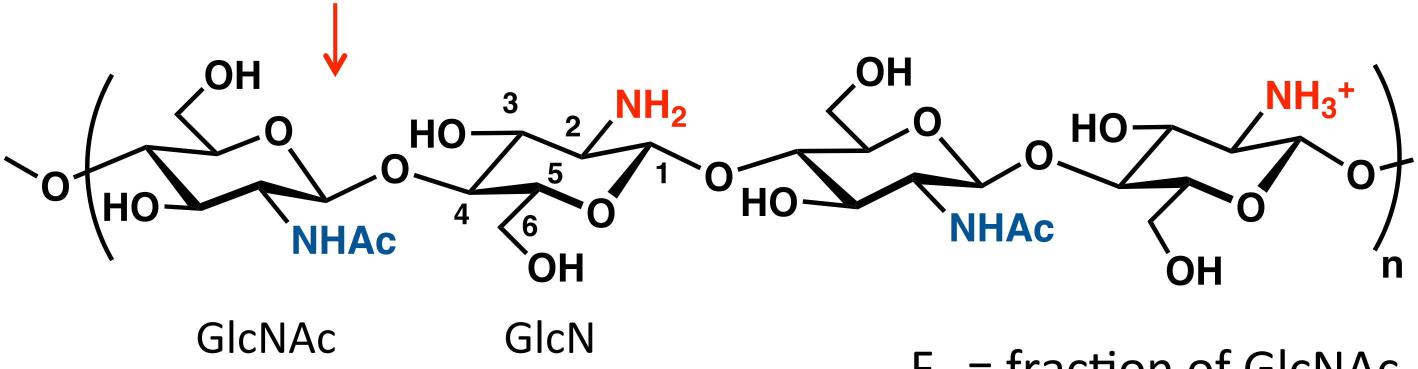
Enzymatic depolymerization of starch



Ismaya et al, DOI: 10.5772/51325. www.intechopen.com/

Cationic polysaccharides chitin and chitosan

- Chitin is the second most abundant organic molecule on Earth
- but only 10k tons produced annually

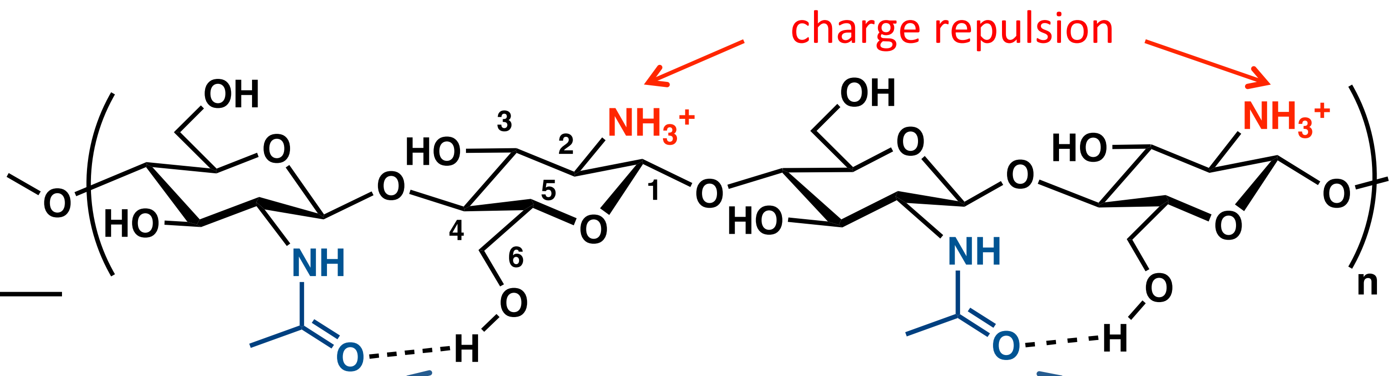

β-(1,4)-glycosidic bond

F_A = fraction of GlcNAc
chitin …….. $F_A = 1$
chitosan…. $F_A = 0 – 0.4$

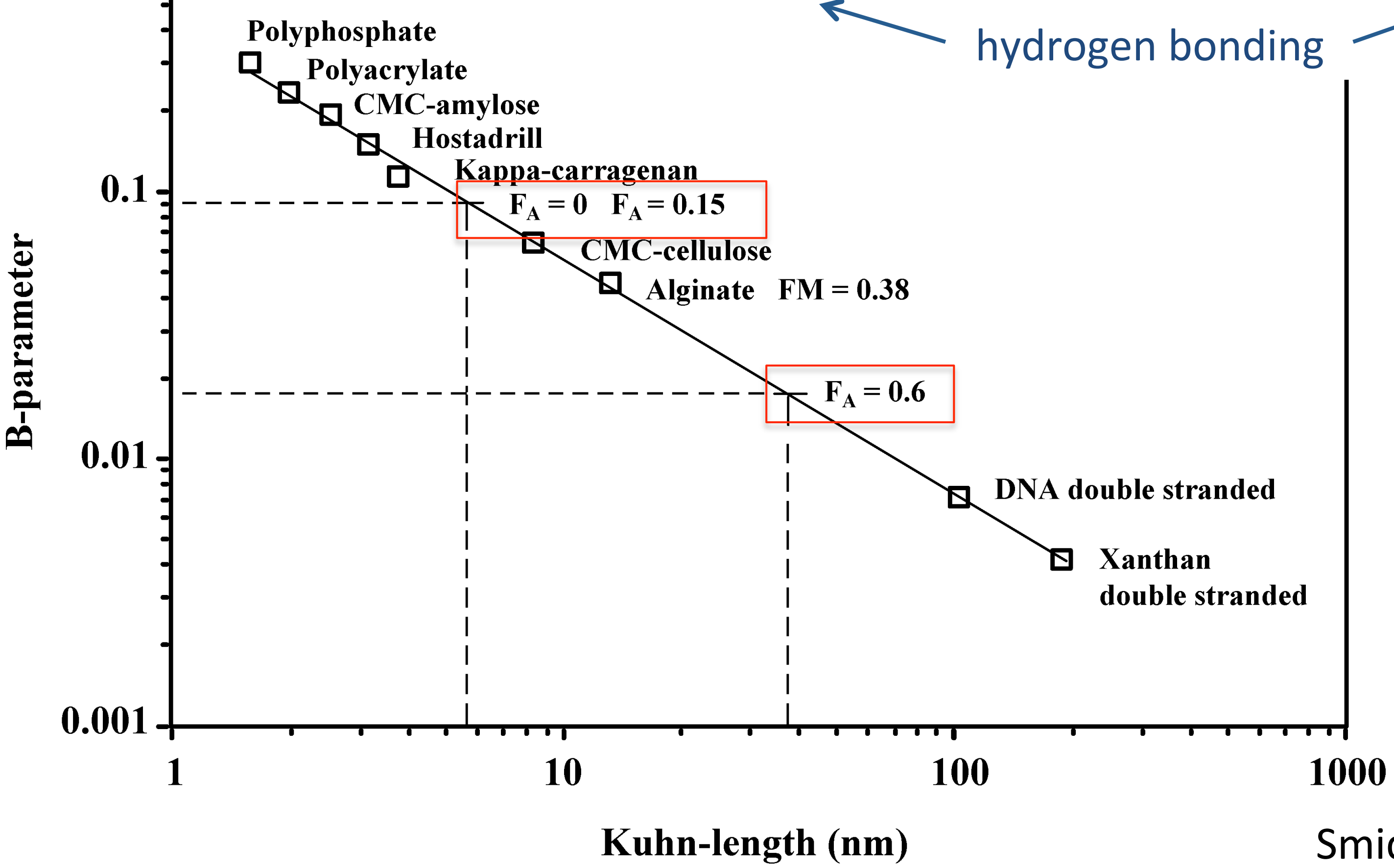
- Copolymers of glucosamine (GlcN) and *N*-acetylglucosamine (GlcNAc)
- Acetyl groups provide hydrophobic character
- Amino groups (especially when protonated) impart hydrophilic character
- Chitosan is an amphiphilic polymer

Stiffness of chitosan is a function of F_A

charge repulsion



hydrogen bonding



Smidsrød and Christensen

Affinity of chitosan for metal ions

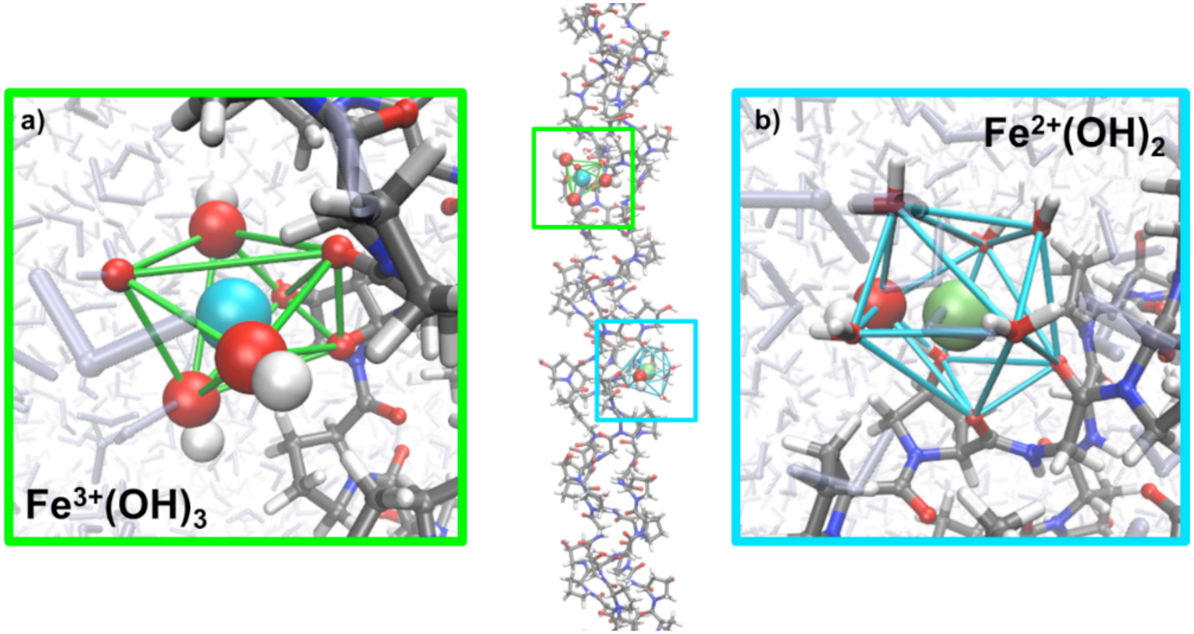
- Selectivity of chitosan toward metals:

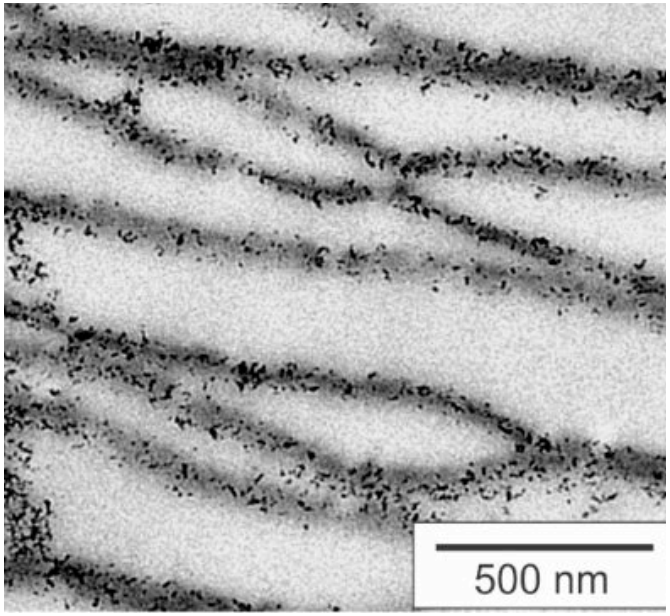
Cu > Hg > Zn > Cd > Ni > Co = Ca

Applications in: floculation/water treatment

biomineralization and fabrication of hybrid nanomaterials

<u>magnetic nanoparticle/chitin composite:</u>





Siglreitmeier *et al. Beilstein J. Nanotechnol*. 2015, 6, 134–148.

Applications of chitosan in wound healing and drug delivery

- Polycationic chitosan can serve as a bioadhesive and has coagulant properties.

- Syvek – trademarked material for surgical sutures and pads.

- Biodegradability and drug loading can be tuned by optimizing F_A and through chemical modification of free NH_2 groups.

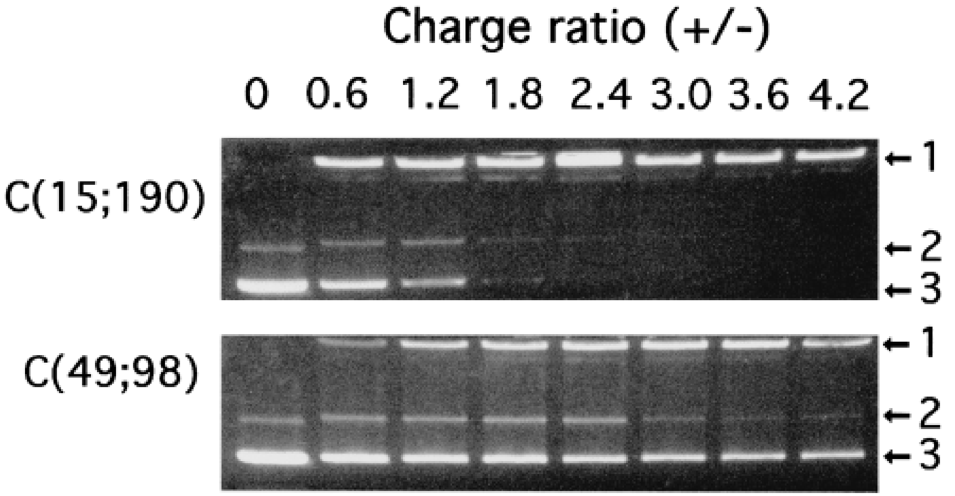
- Drug delivery to negatively charged mucosal membranes

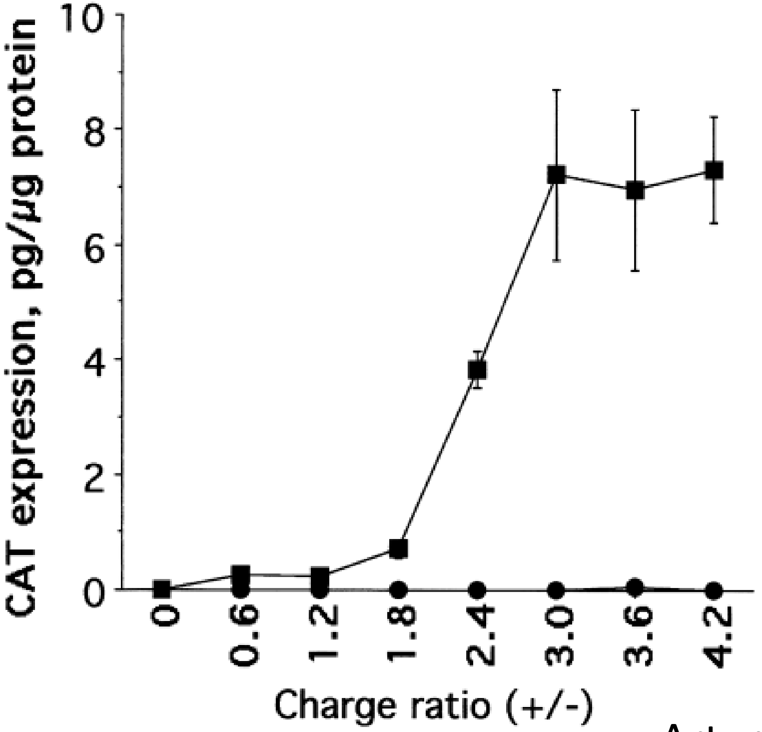
Applications of chitosan in non-viral gene delivery

Chitosan:

C(15, 190): F_A – 0.15, Mw 190 KDa
C(49, 98): F_A – 0.49, Mw 98KDa

increasing charge ratios and lower F_A provide more effective DNA packing



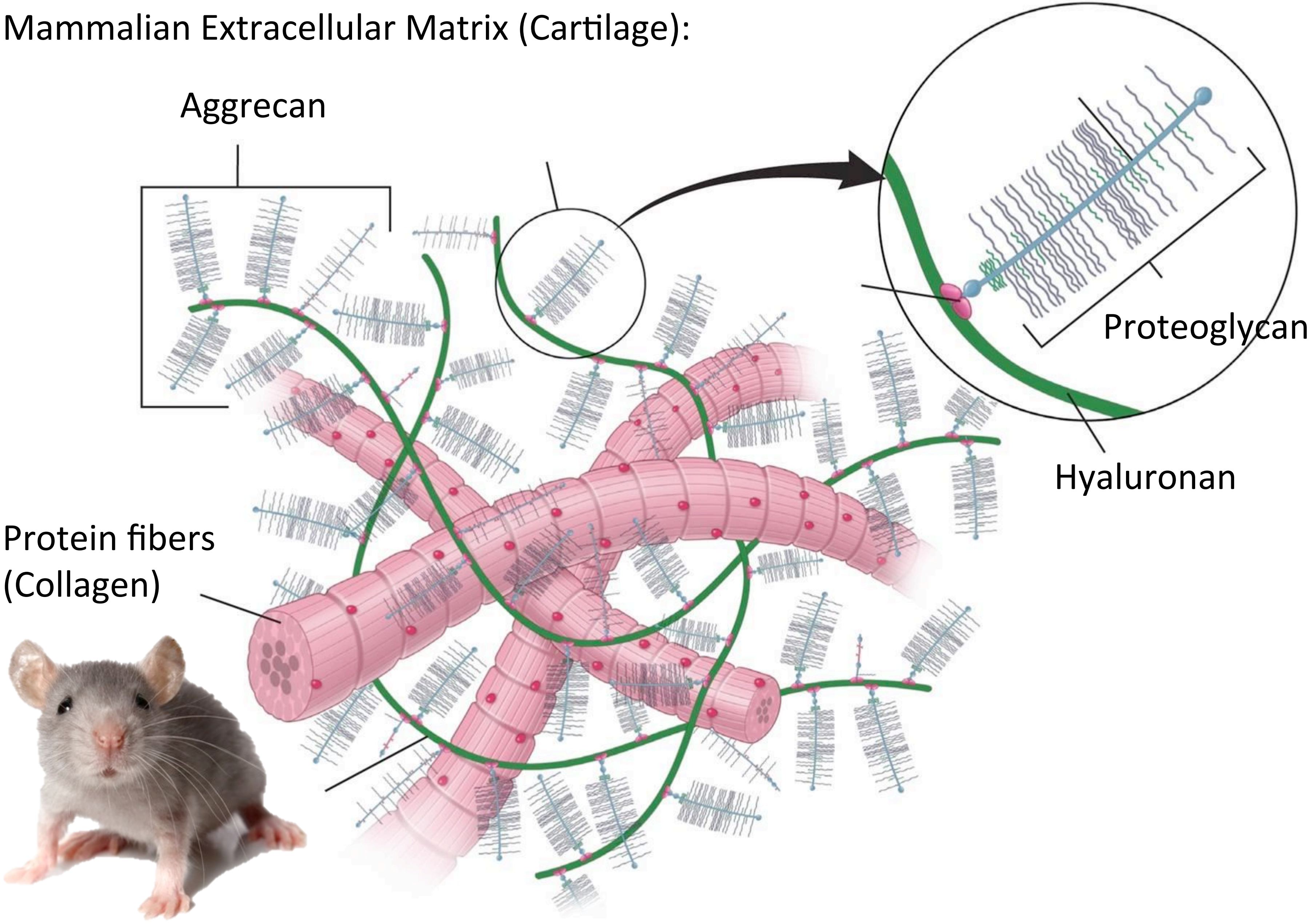


Chitosan with F_A = 0.15 effectively delivers the chloramphenicol acetyl transferase (CAT) gene in HEK293 cells.

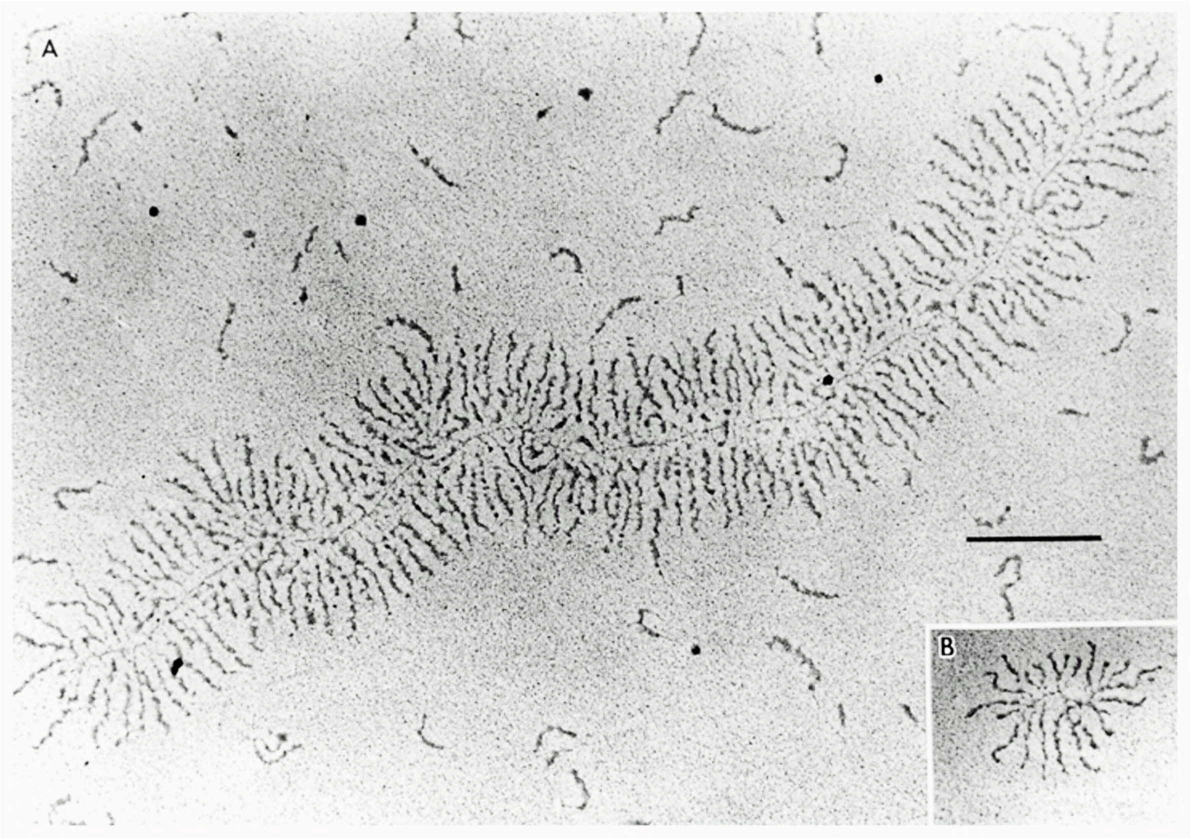
Artursson and co-workers Gene Therapy (2001) 8, 1108

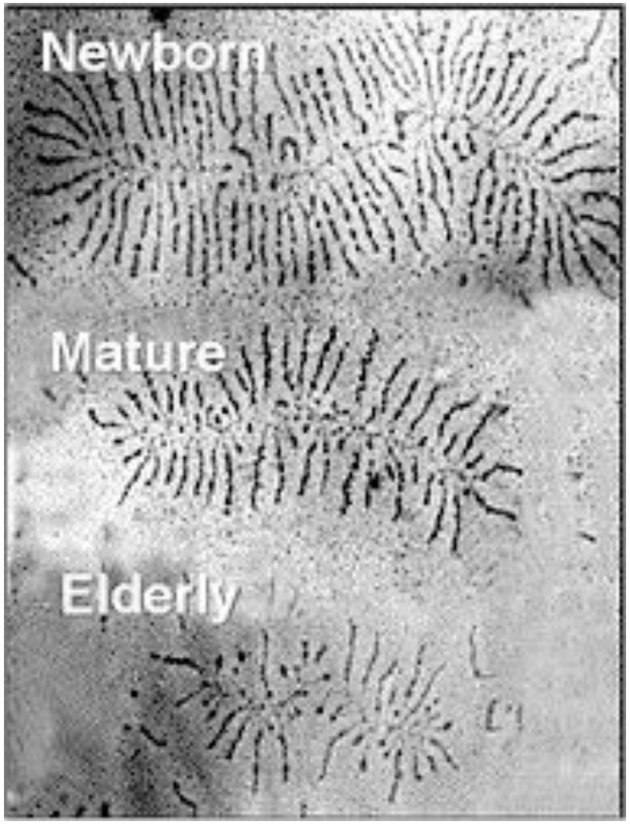
Anionic polysaccharides – glycosaminoglycans

Mammalian Extracellular Matrix (Cartilage):



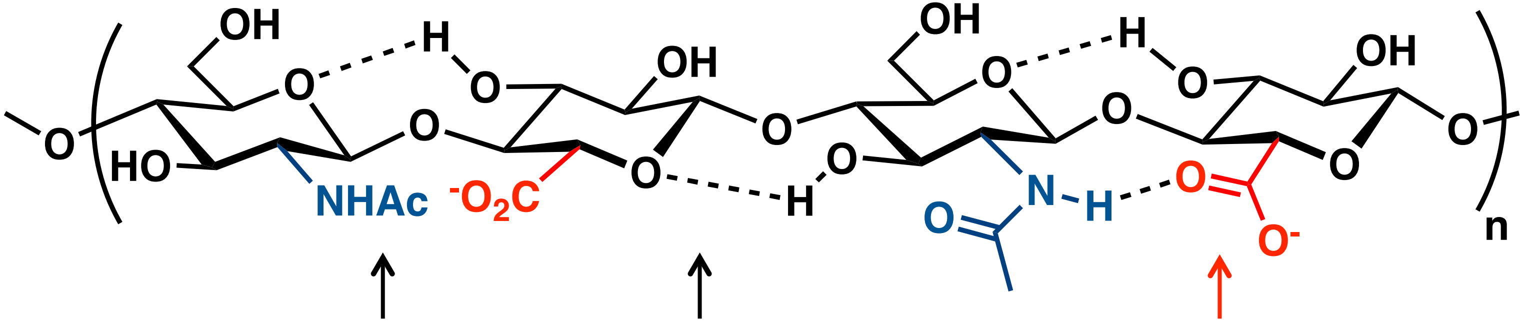
Aggrecan (and our cartilage) degrades with age





Buckwalter *et al Bone Joint Surg Am*, 1997 Apr; 79 (4): 600 -11

Structure of hyaluronic acid (HA)



N-acetylglucosamine (GlcNAc)

glucuronic acid (GlcA)

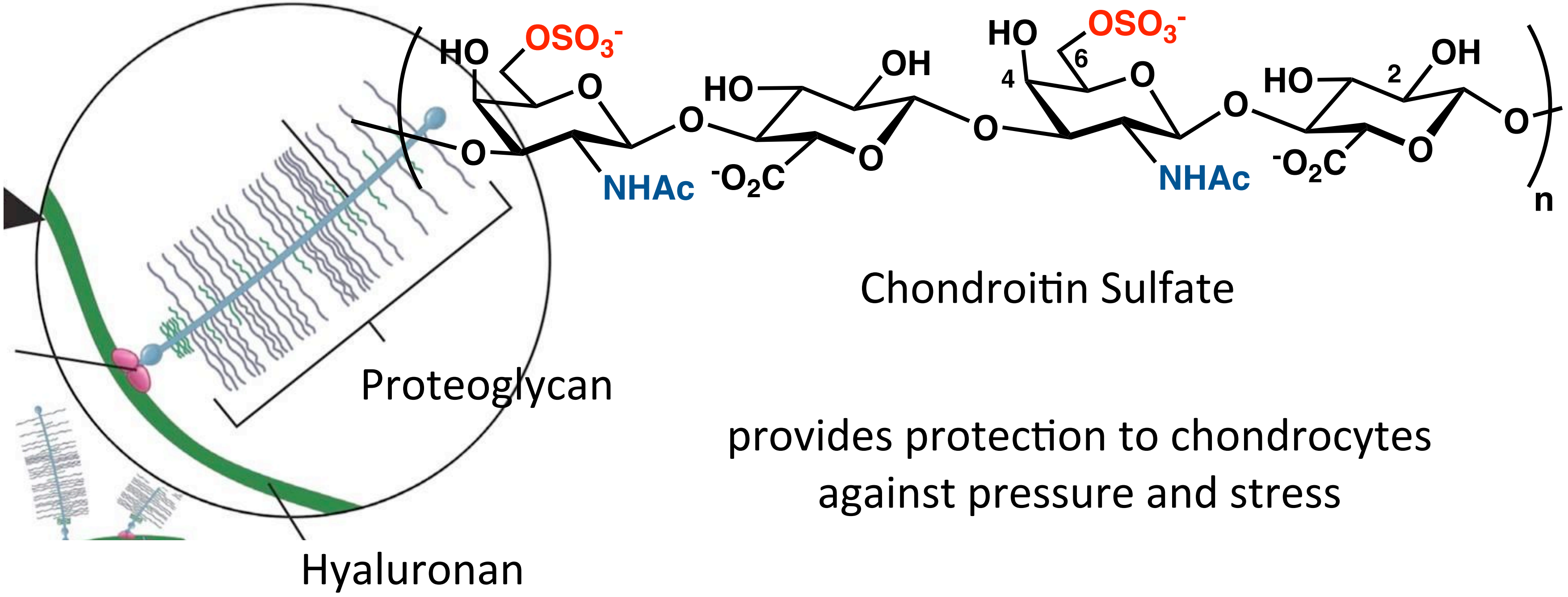
β-(1,4)-glycosidic bonds

charge repulsion provides extended chain conformations

- Extensive intramolecular H-bonding.
- Mw ranges from several kDa to 10’s of MDa
- Biological activity of HA depends on Mw (or DP).
- Low Mw HA fragments have pro-inflammatory properties.
- High Mw HA provides mechanical support and water homeostasis in tissues.
- Tumor cells secrete hyaluronidase enzymes to break down the matrix and allow for their invasion and tumor growth.

Sulfated glycosaminoglycans

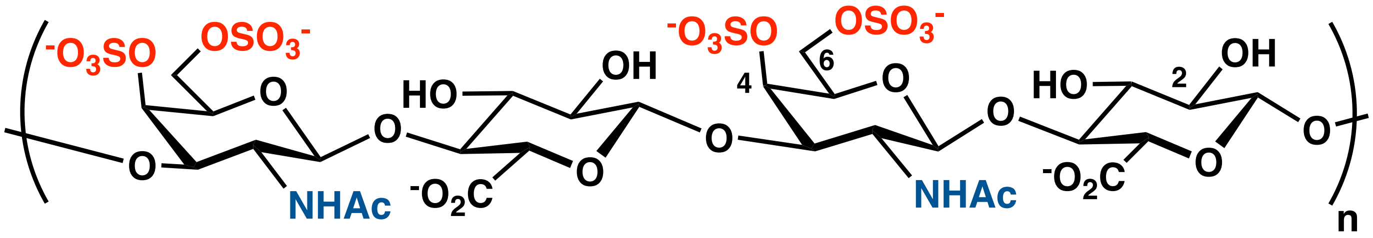
cartilage:



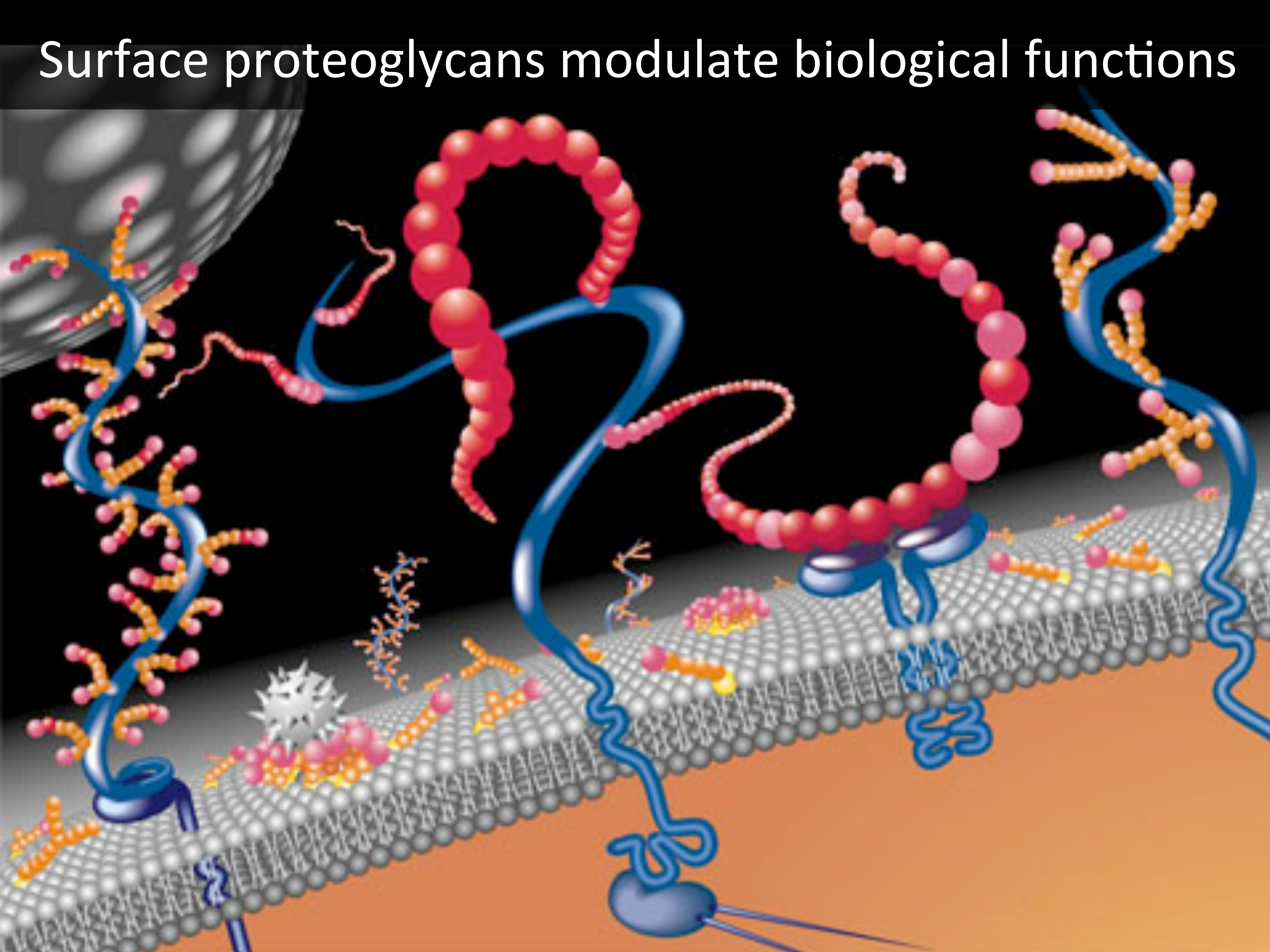
Chondroitin Sulfate

provides protection to chondrocytes against pressure and stress

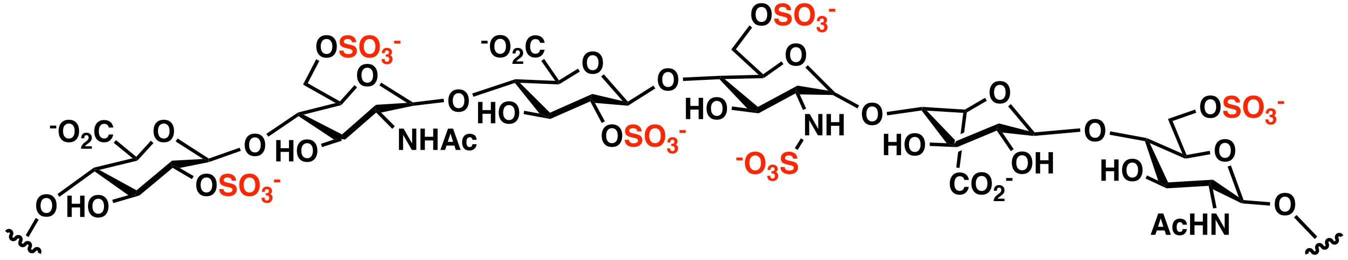
In neural tissues, CS either promotes or inhibits neural growth depending on sulfation pattern:



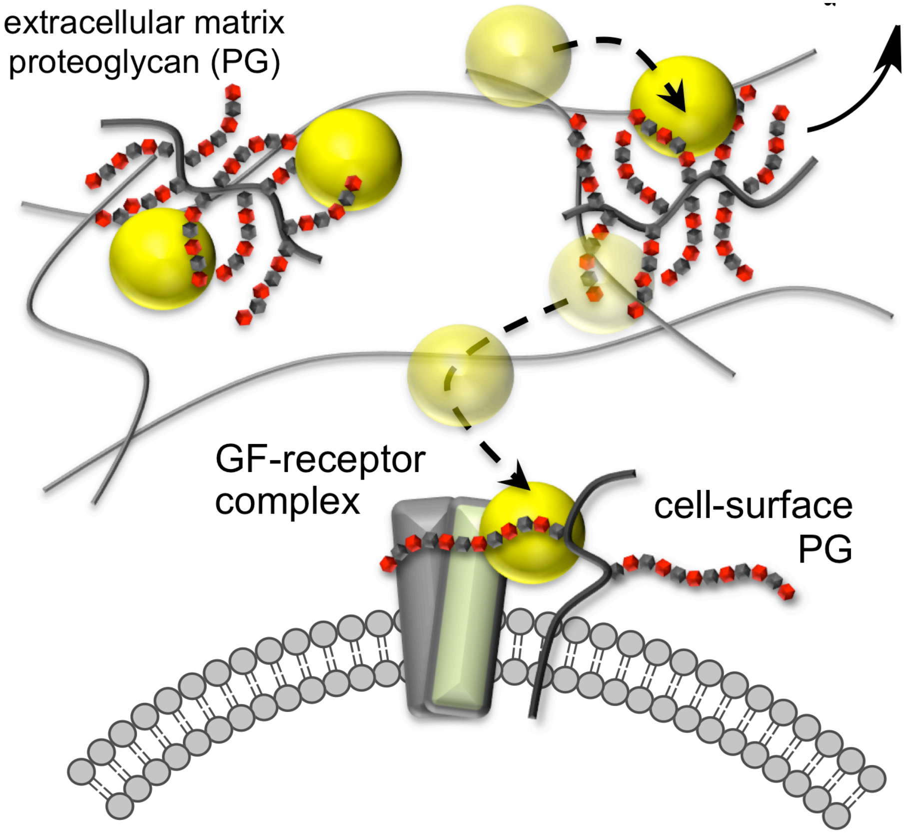
Surface proteoglycans modulate biological functions



Proteoglycans, through their glycosaminoglycan chains, regulate growth factor signaling



heparan sulfate glycosaminoglycan
(HS GAG)



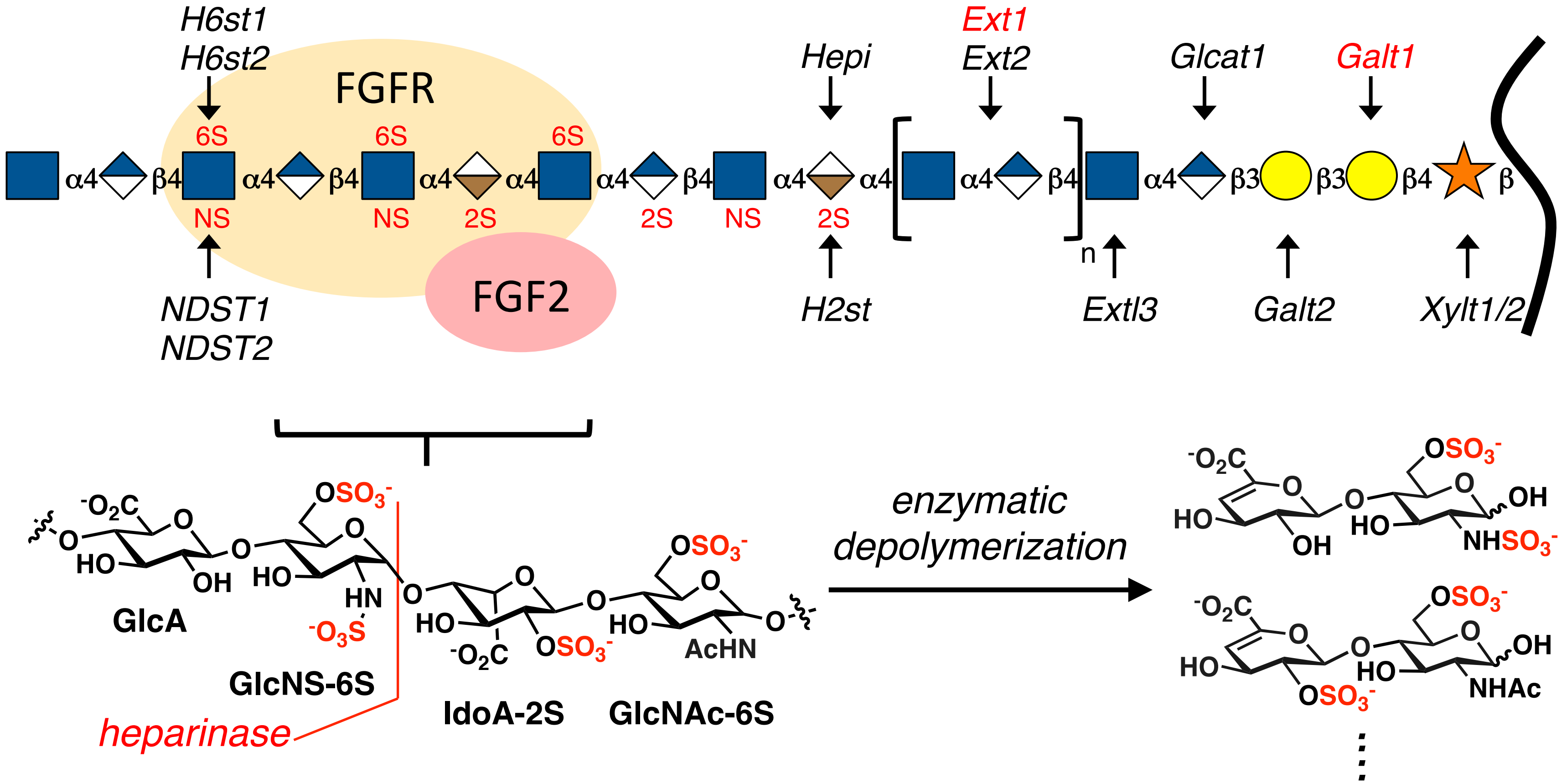
extracellular matrix
proteoglycan (PG)

GF-receptor
complex

cell-surface
PG

- GAGs control presentation and delivery of growth factors and other morphogens
- Sulfate patterns span several nanometers and encode protein binding motifs

Sequences of HS glycans encode binding information



heparan sulfate (HS) glycosaminoglycan

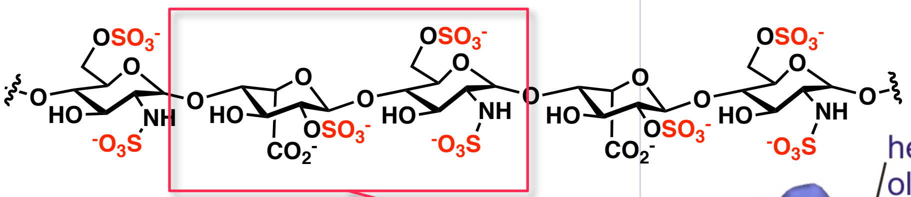
diGAG library

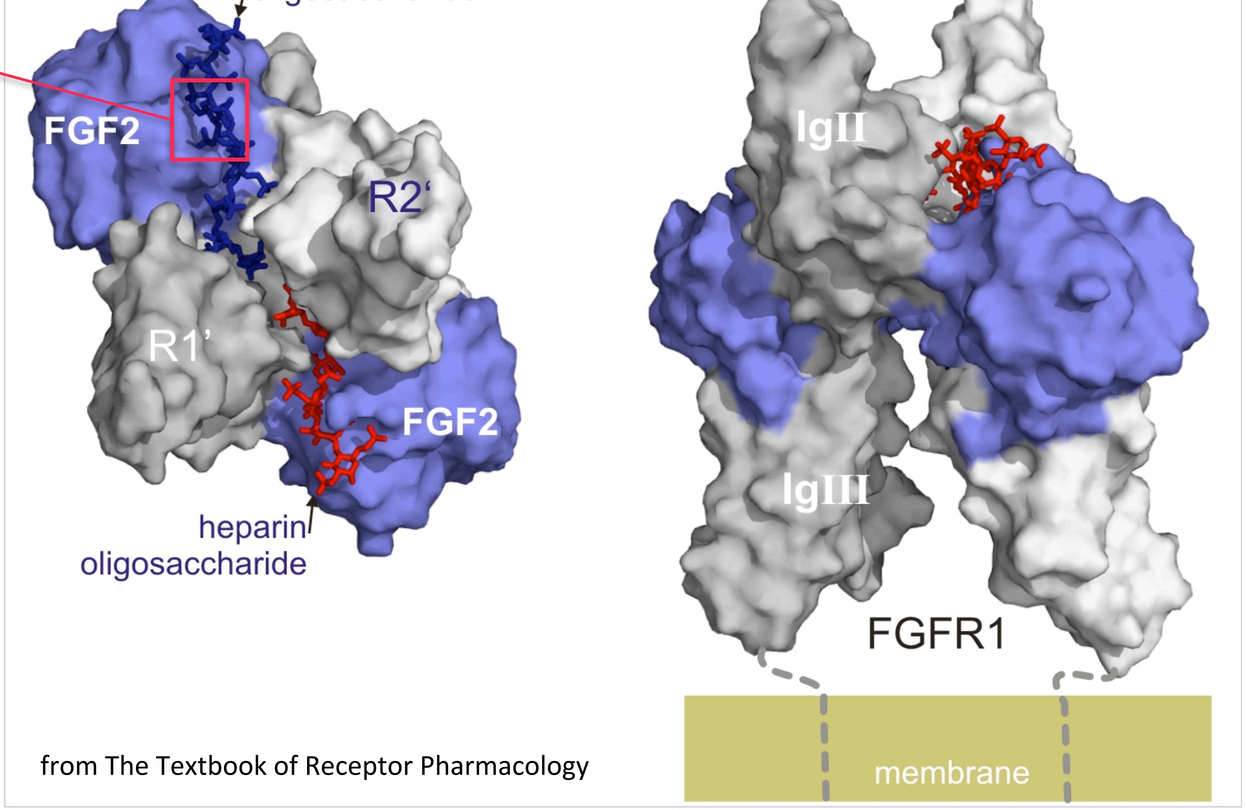
■ *N-Acetylglucosamine (GlcNAc)* ◆ *Glucuronic Acid (GlcA)* ◇ *Iduronic Acid (IdoA)* ● *Galactose (Gal)* ★ *Xylose (Xyl)* S *= sulfate*

Figure stolen from Esko

GAG on proteoglycans organized receptor complexes

heparan sulfate GAG





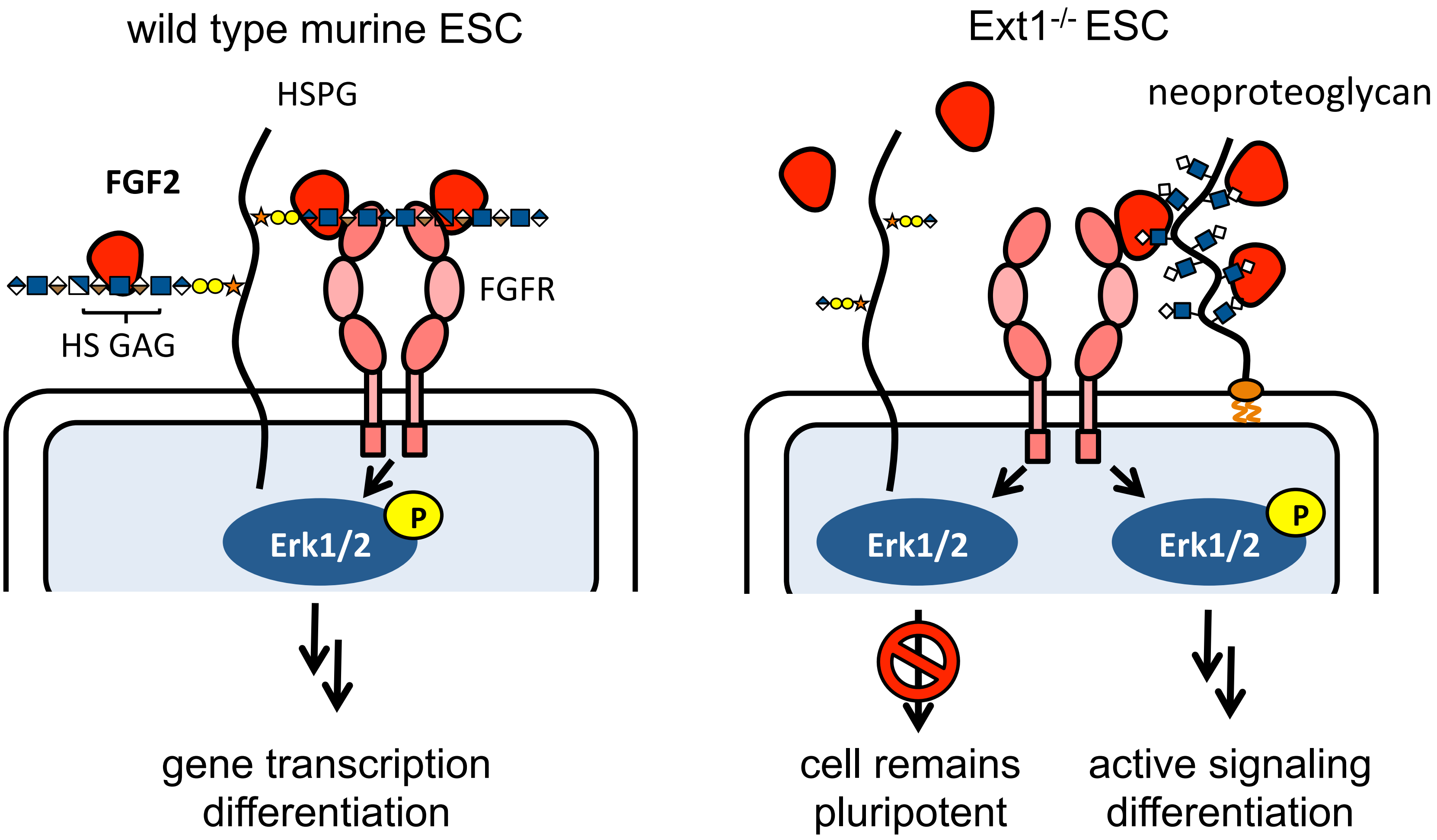
from The Textbook of Receptor Pharmacology

Schlessinger *et al Mol. Cell* (2000)

- Unique sulfation patterns may be necessary for specificity of signaling
- Nanoscale presentation of sulfate patterns is required for proper function

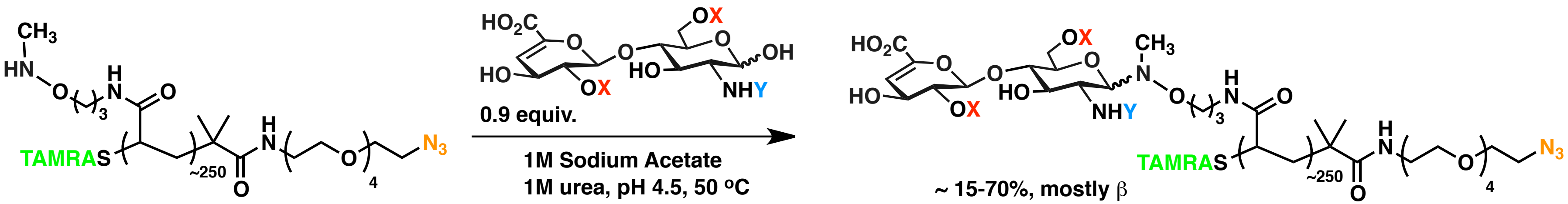
Yayon *et al Cell* (1991)
Rapraeger *et al Science* (1991)

HS GAGs are essential in vertebrate development



Johnson *et al Stem Cells 25*, 1913 (2007)
Kraushaar, Yamaguchi, and Wang *J. Biol. Chem.* (2010)

Synthesis of neoproteoglycans and their interactions with FGF2

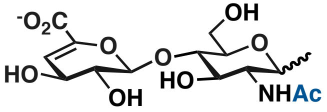


0.9 equiv.

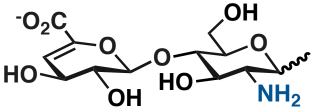
1M Sodium Acetate
1M urea, pH 4.5, 50 °C

~ 15-70%, mostly β

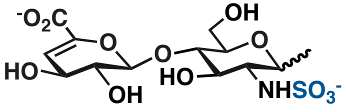
X = H or SO_3^-, Y = H, Ac or SO_3^-



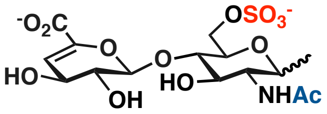
A, D0A0



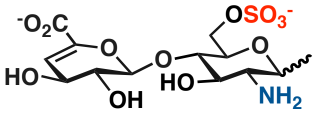
E, D0H0



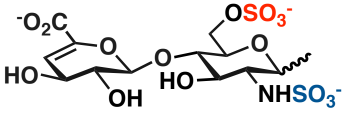
I, D0S0



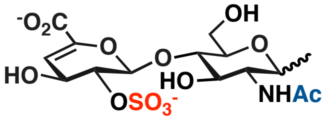
B, D0A6



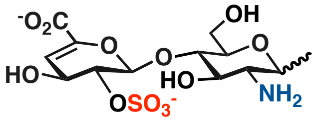
F, D0H6



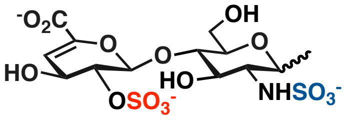
J, D0S6



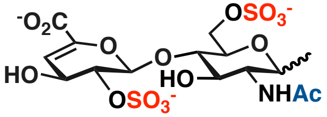
C, D2A0



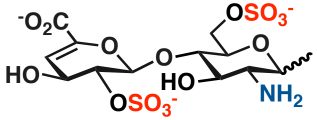
G, D2H0



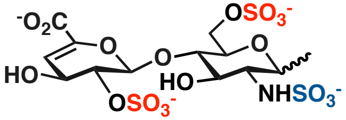
K, D2S0



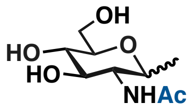
D, D2A6



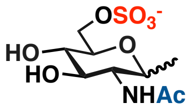
H, D2H6



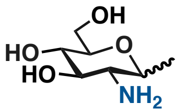
L, D2S6



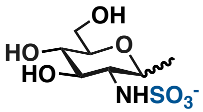
M, GlcNAc



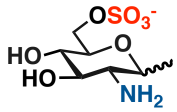
N, GlcNAc-6S



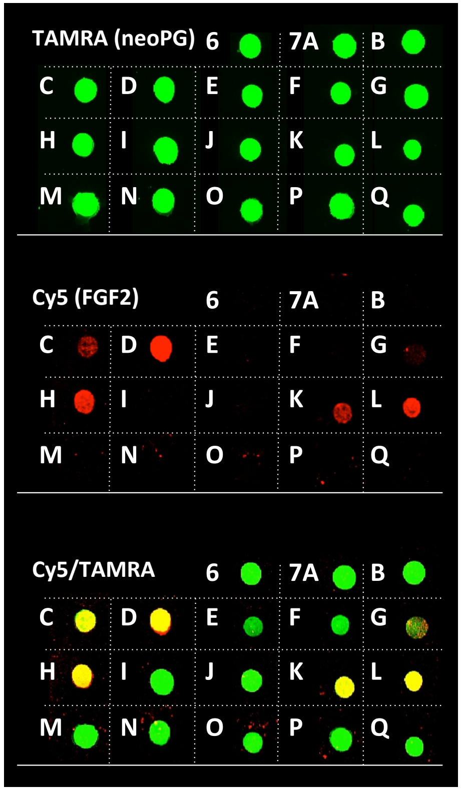
O, GlcN



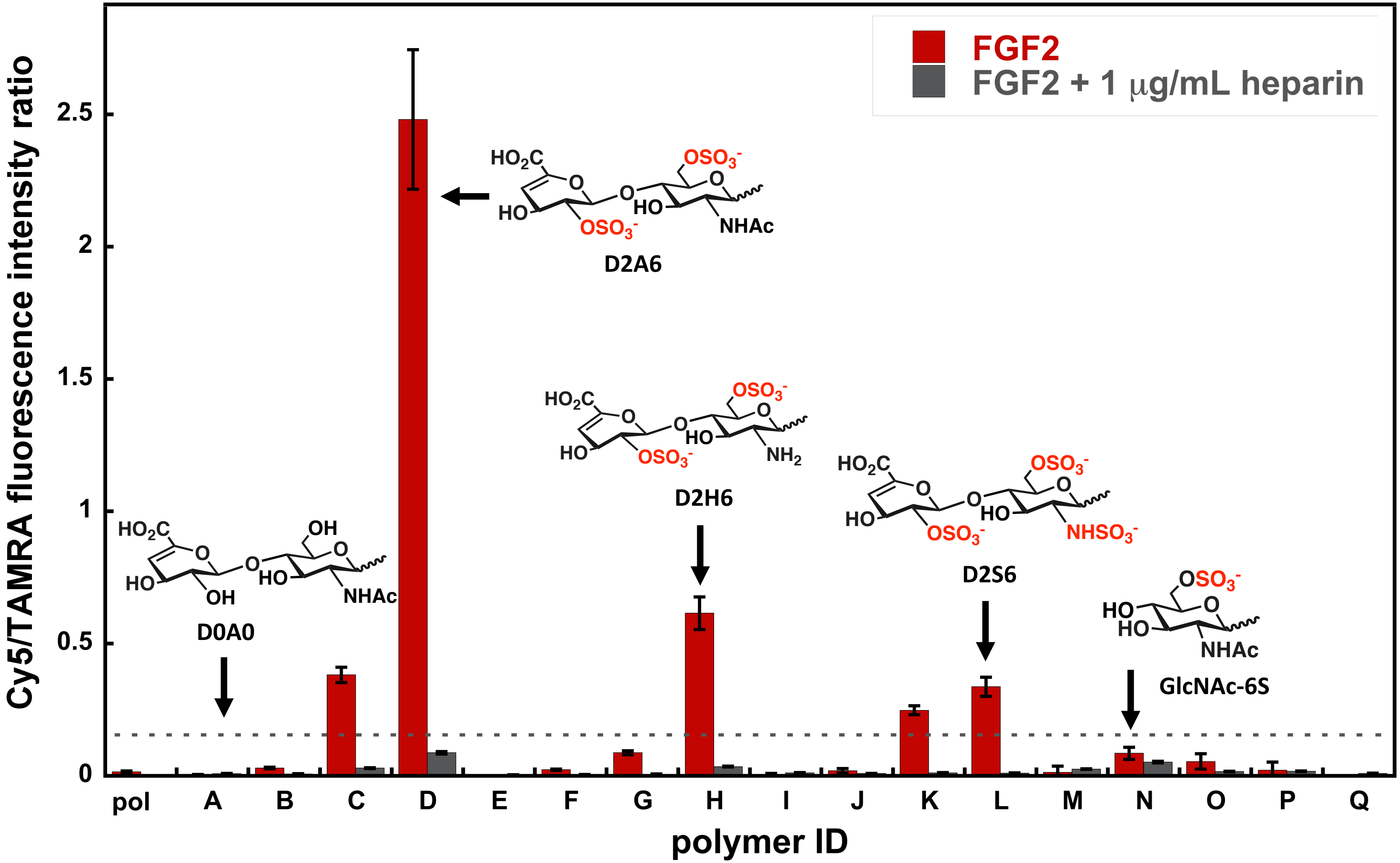
P, GlcN-2S



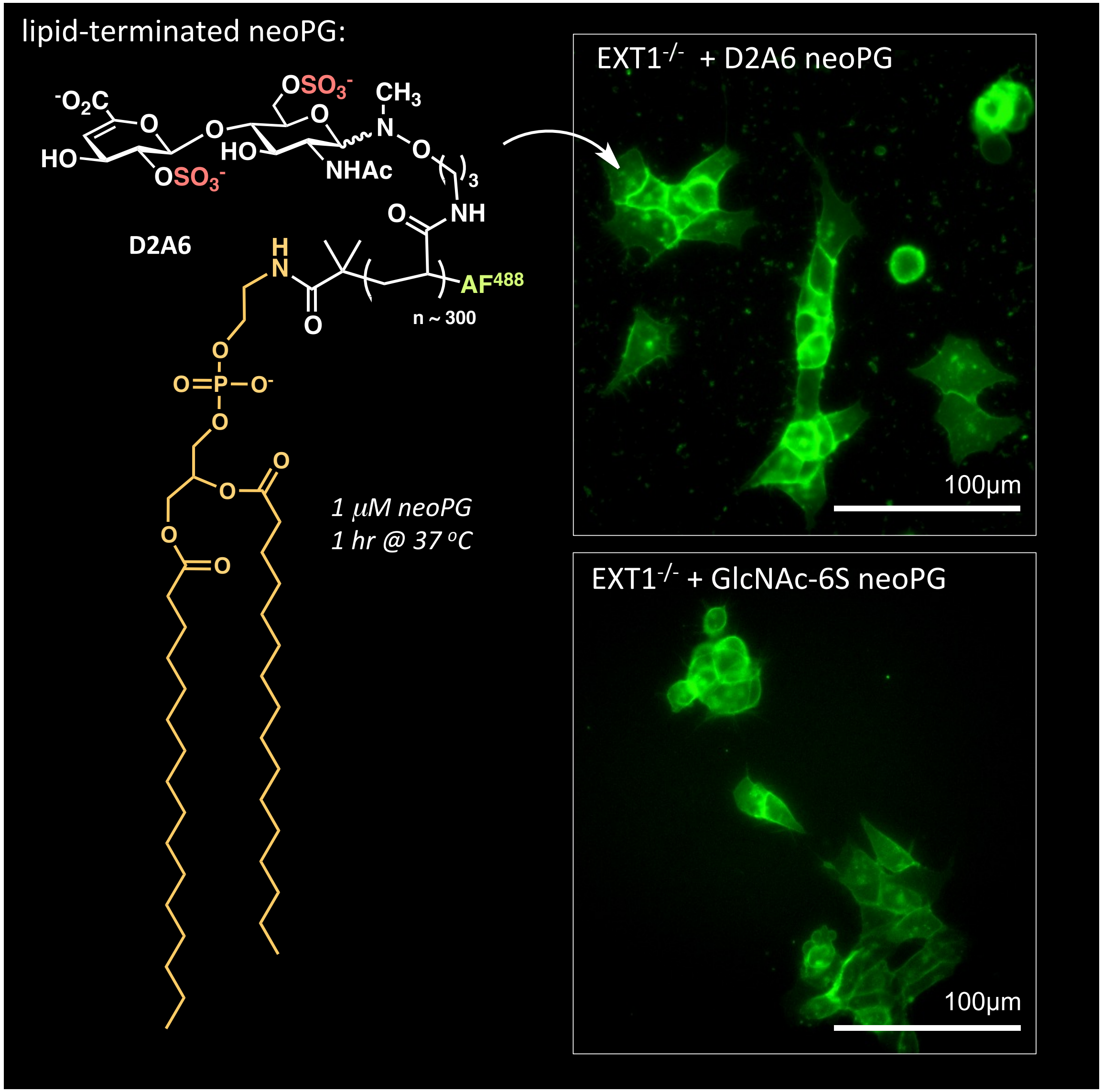
Q, GlcN-6S



Neoproteoglycans engage FGF2 in a sulfate-dependent manner

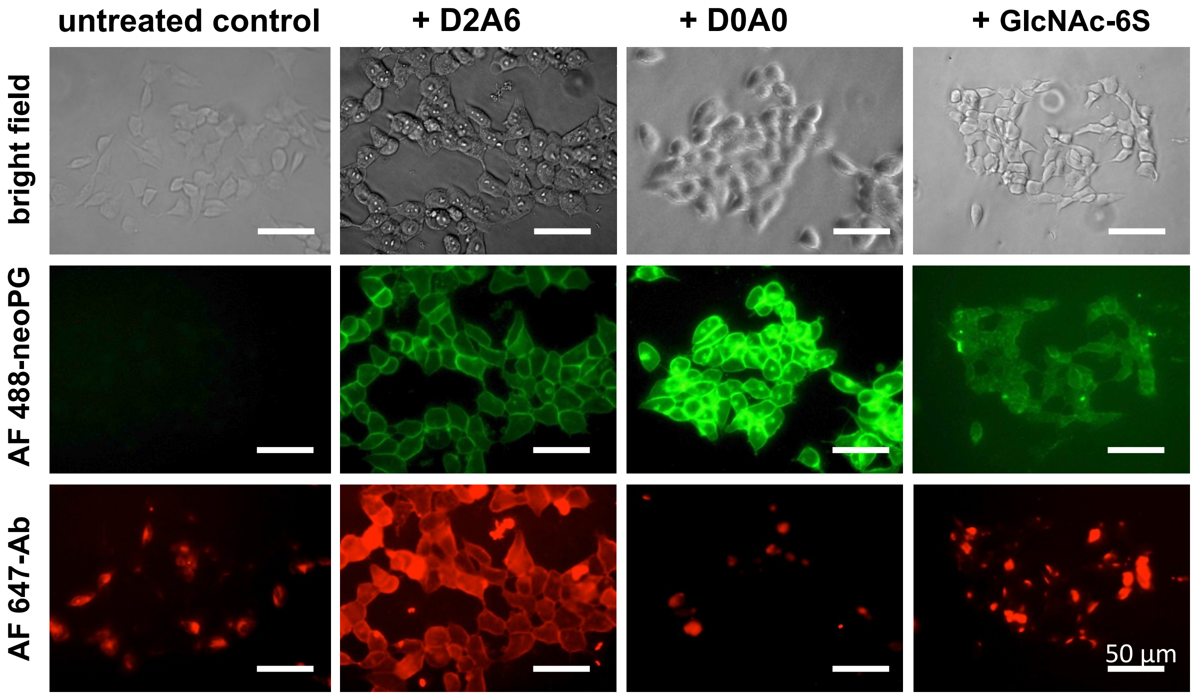


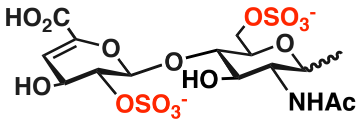
Lipid-terminated neoproteoglycans remodel the glycocalyx of *Ext1$^{-/-}$* mESCs



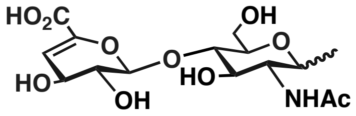
1 µM neoPG
1 hr @ 37 °C

Neoproteoglycans enhance FGF2 binding and activate Erk1/2 phosphorylation

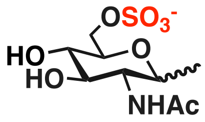




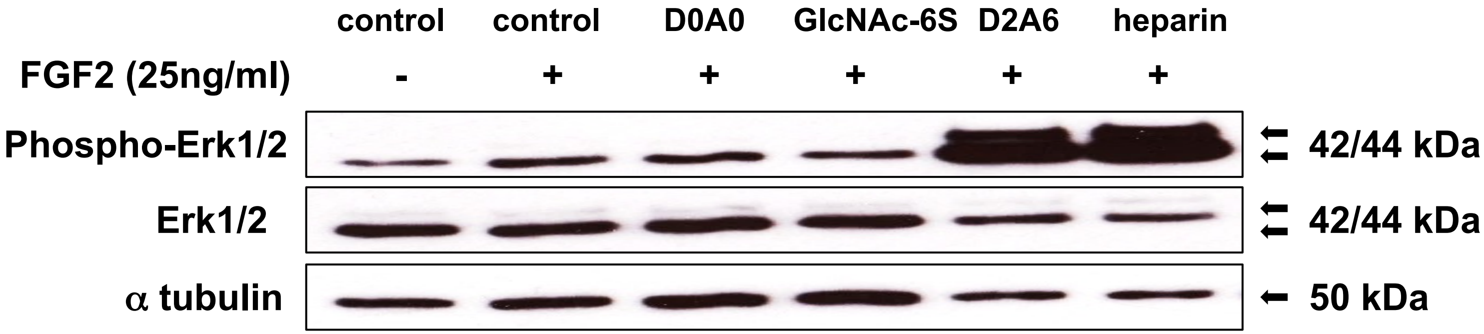
D2A6



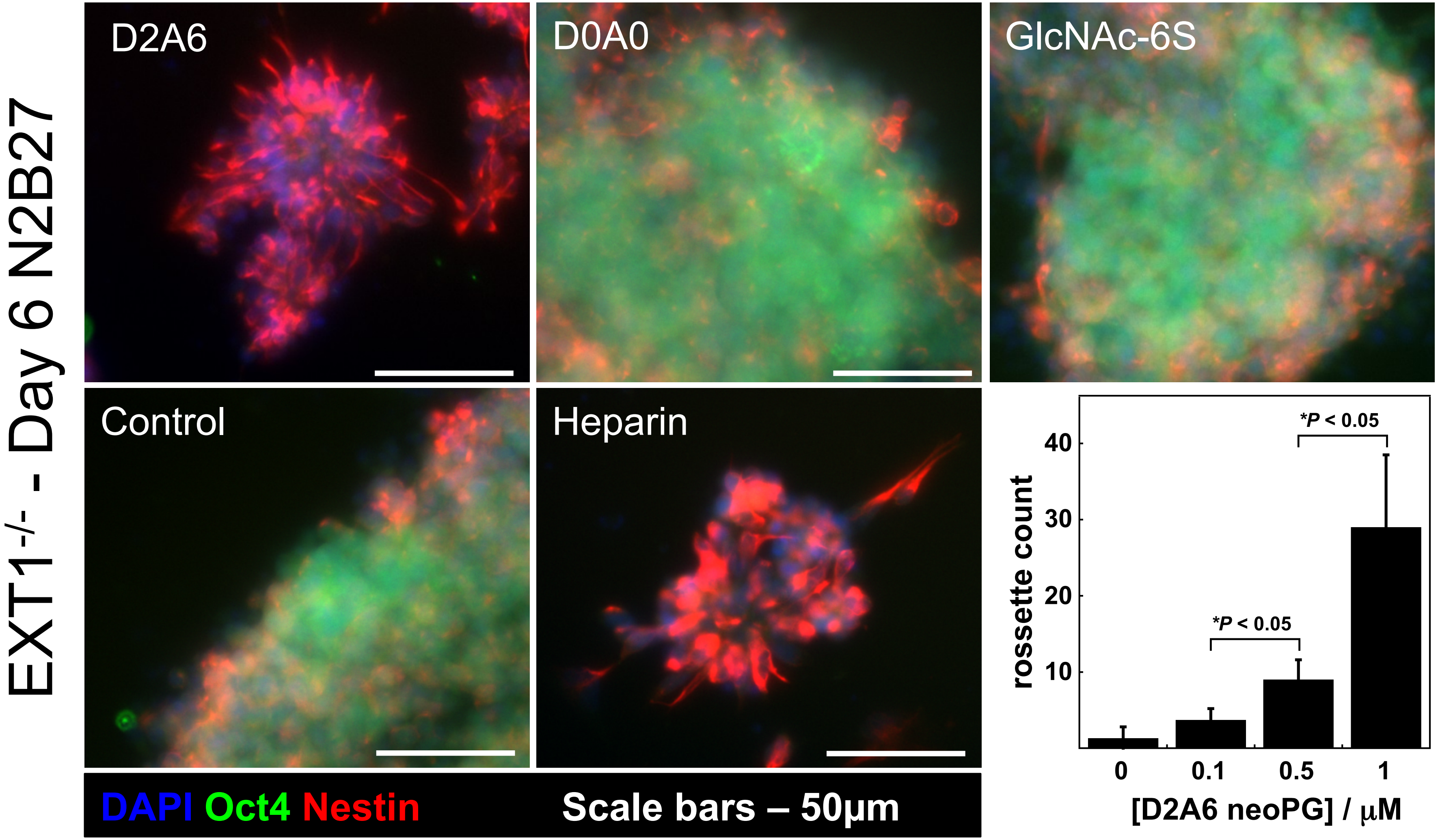
D0A0



GlcNAc-6S

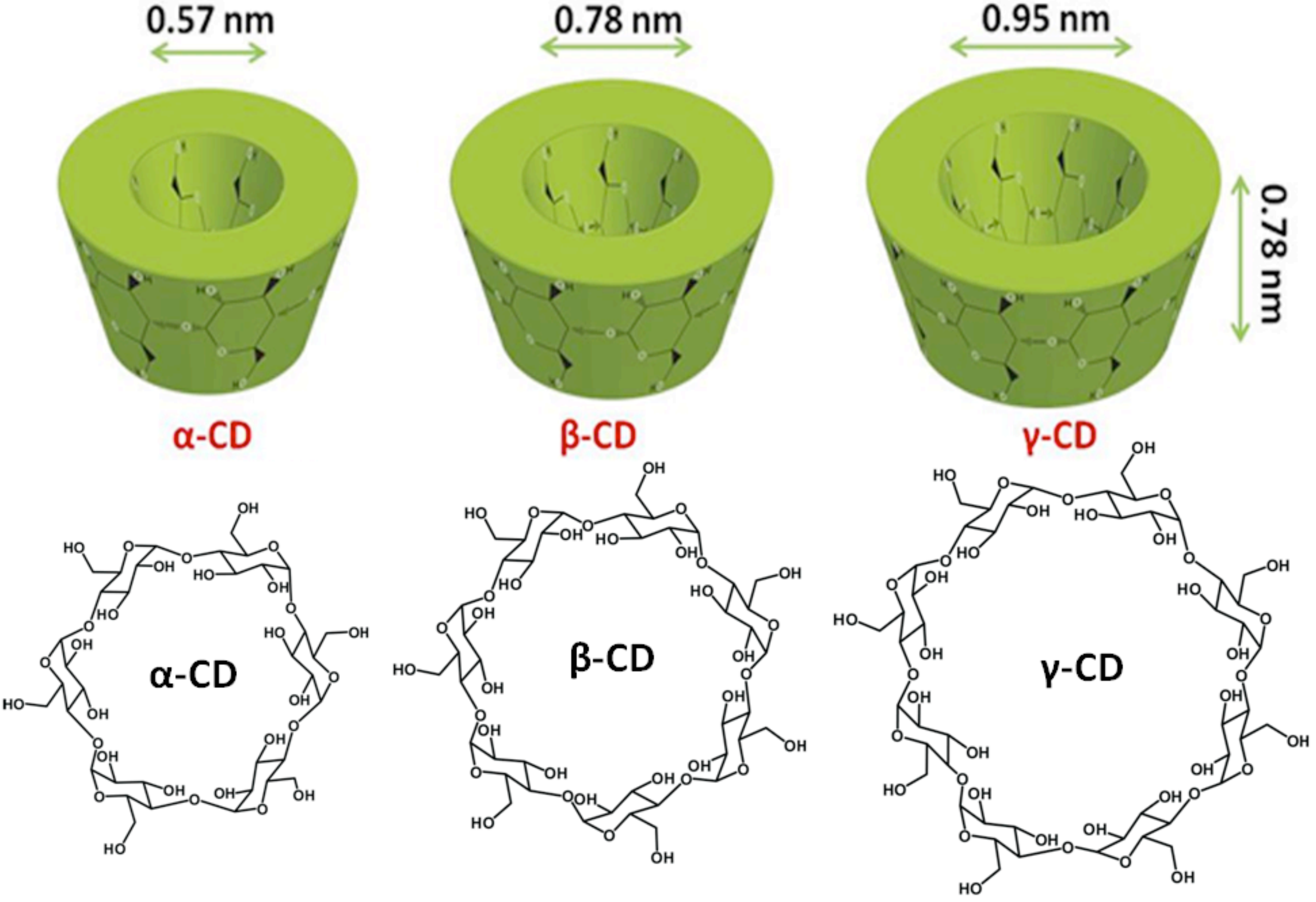


Neoproteoglycans induce neural differentiation



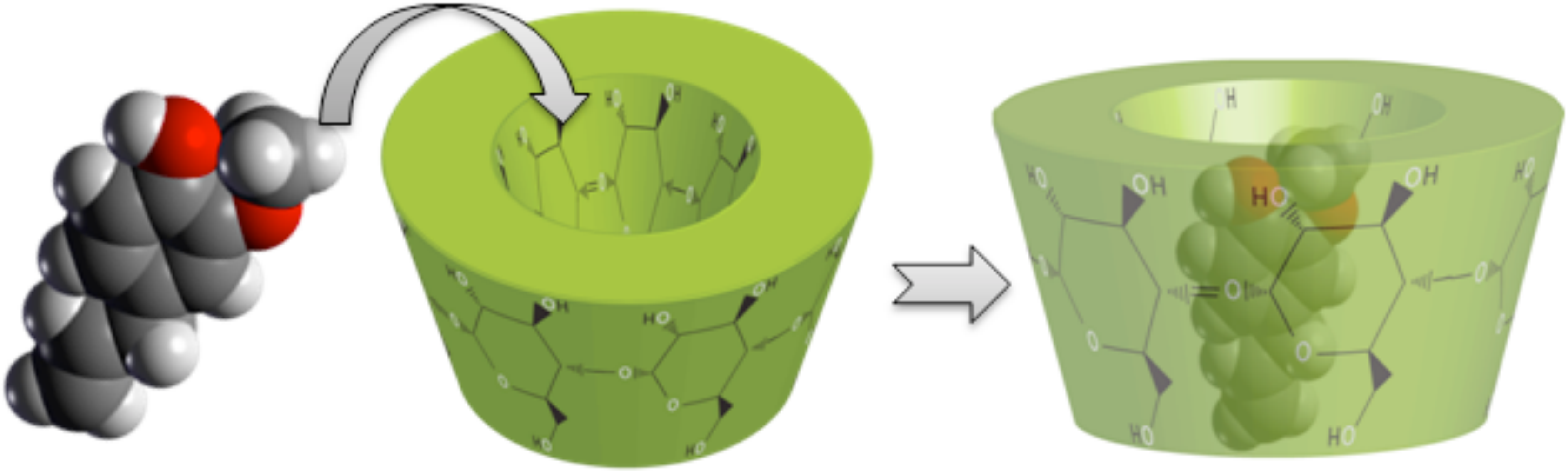
Huang, Smith, Trieger, Godula *JACS* (2014)

Cyclodextrins - cavitands with tunable sizes



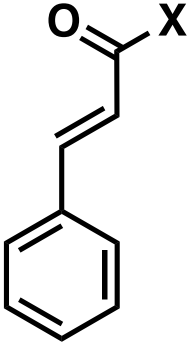
http://unam.bilkent.edu.tr

Cyclodextrins form inclusion complexes

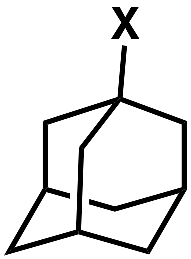


http://unam.bilkent.edu.tr

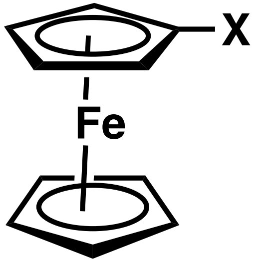
guest molecules:



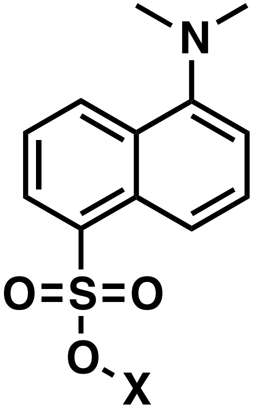
cynamyl



adamantane

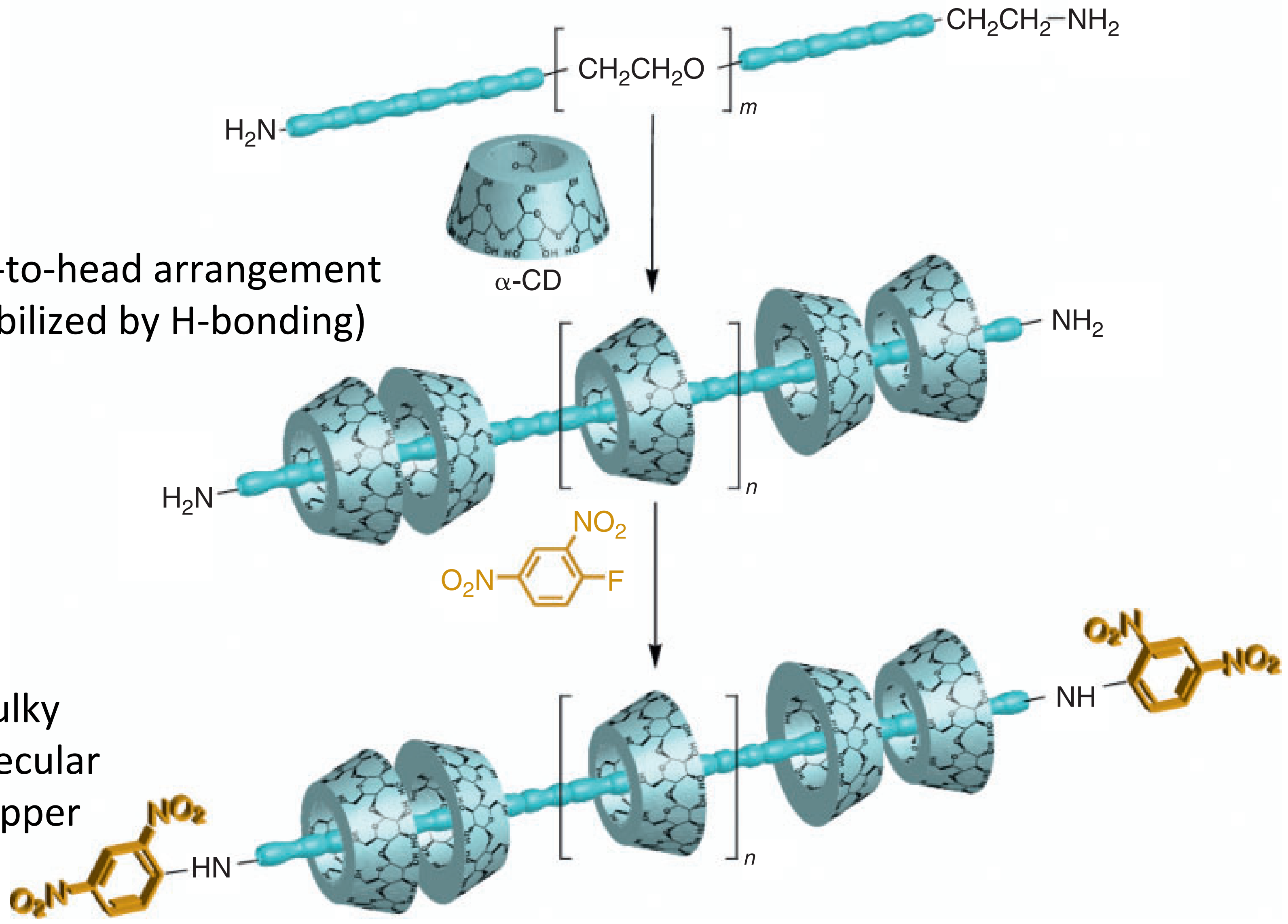


ferrocene



dansyl

Polymers can be threaded through the cavity of CDs

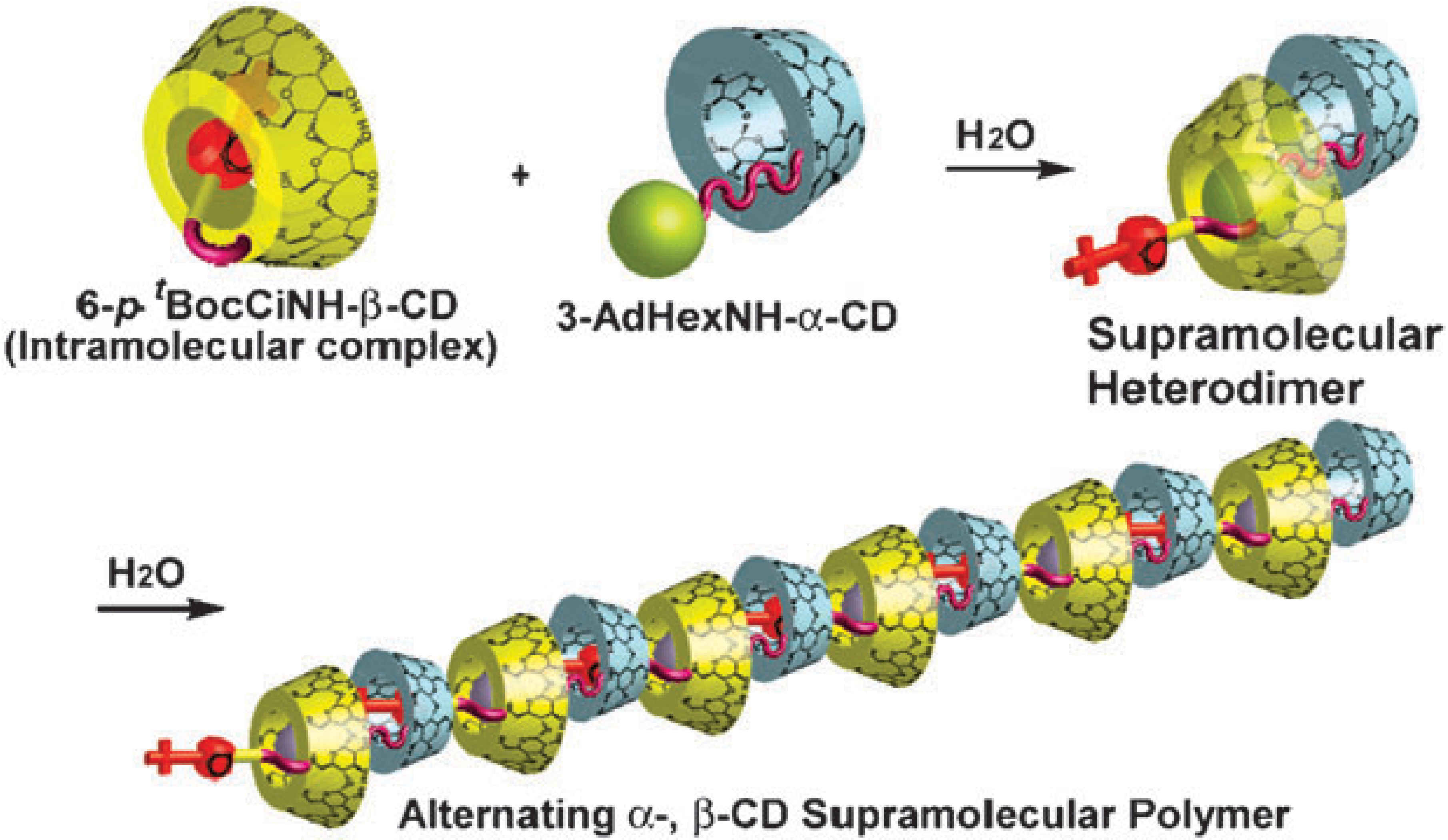


head-to-head arrangement
(stabilized by H-bonding)

bulky
molecular
stopper

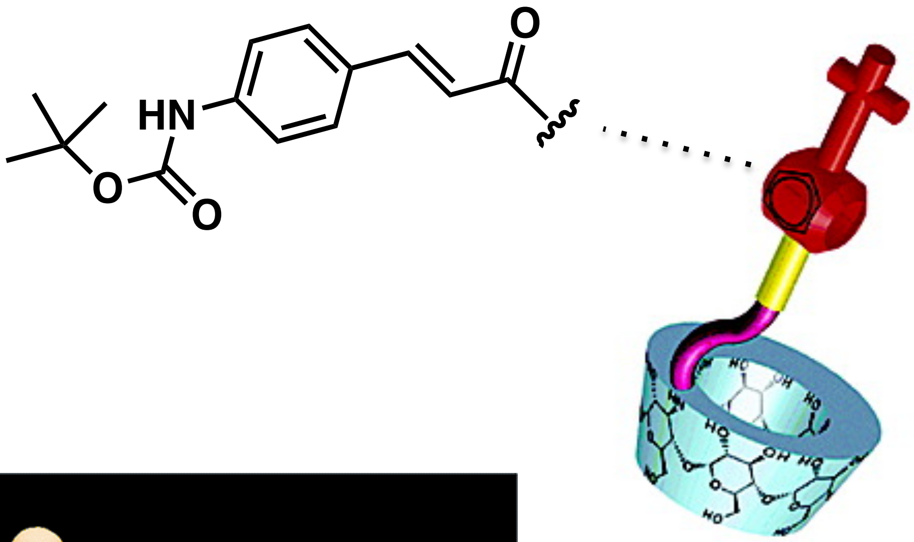
Harada *et al Chem. Soc. Rev.*, 2009,38, 875-882

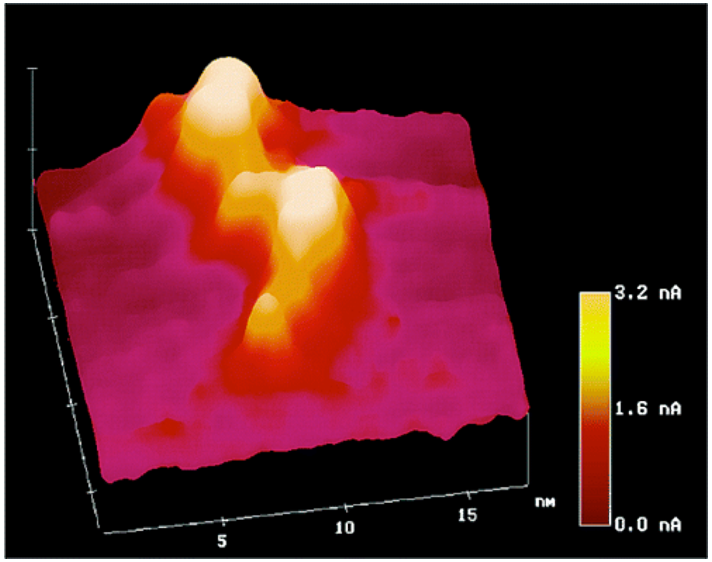
α,β-Alternating supramolecular polymers of cyclodextrin



Harada *et al Chem. Soc. Rev.*, 2009,38, 875-882

Helical supramolecular cyclodextrin polymers





Masahiko *et al J. Am. Chem. Soc.* **2005,** 127, 2984-2989.