Binding of hyaluronan and its neutral analog by TSG-6 Link domain

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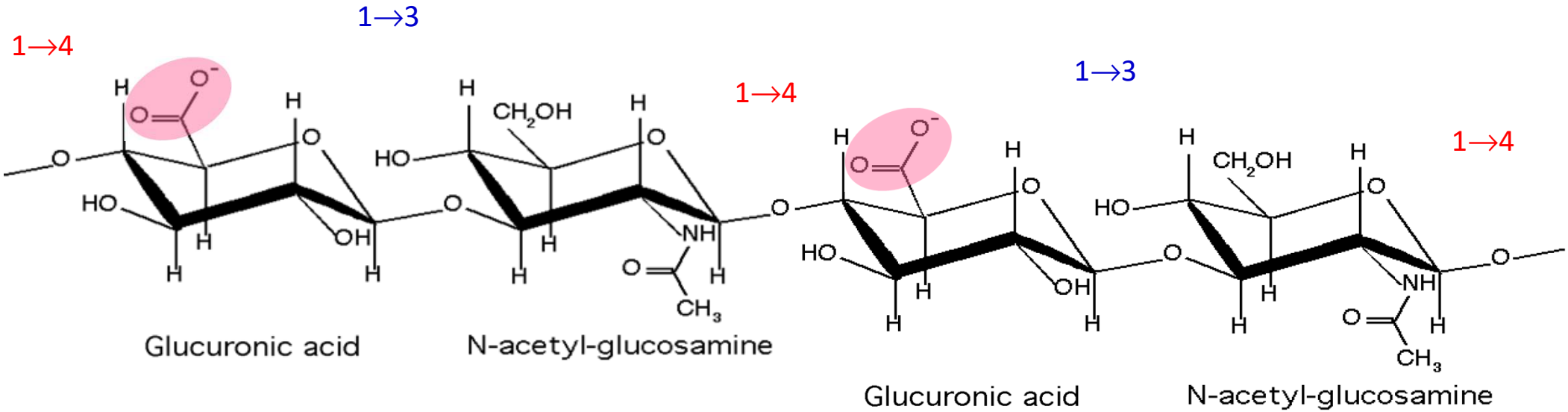
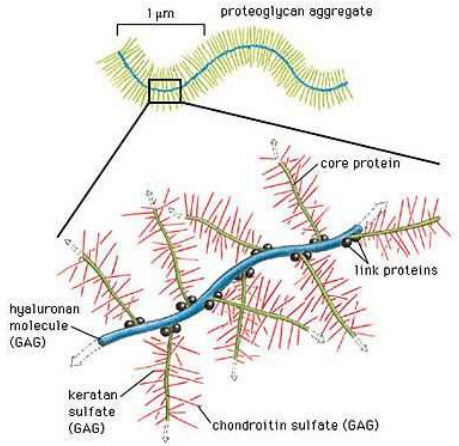
Hyaluronic acid (hyaluronan) – **HA**

[4)-β-D-Glc*p*A-(1–>3)-β-D-Glc*p*NAc-(1–>]$_n$



- Essential component of proteoglycan - the main constituent of the extracellular matrix of connective tissues
- Signaling molecule involved in carcinogenesis, inflammation and wound healing
- Biological activity depends on the chain length (from MDa to short oligosaccharides)



http://medinfo.ufl.edu/pa/chuck/summer/handouts/images/gag.jpg

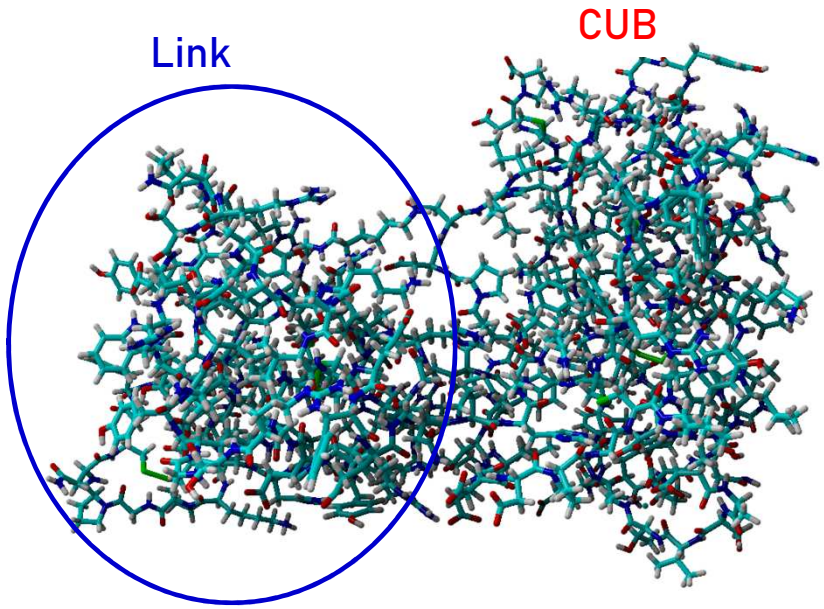
Hyaladherins – hyaluronan binding proteins

- Containing Link domain
  - Proteoglycan forming
    - Aggrecan
    - Brevican
    - Neurocan
    - Versican
  - Membrane receptors
    - CD44 – the main transmembrane receptor
    - LYVE-1 – lymphatic endothelial cells
  - Soluble receptor
    - TSG-6
- Without Link domain
  - RHAMM – membrane receptor, partially disordered
  - Inter-α-inhibitor

TSG-6

Consists of Link and CUB domain

- Link
  - binds hyaluronan and other GAGs
  - positively charged
- CUB
  - not well known function
  - negatively charged



Interactions of TSG-6 with glycosaminoglycans (GAGs)

Several interactions of GAG oligosaccharides were identified by NMR experiments

Hyaluronan[1]: K11, Y12, H45, V57, Y59, P60, I61, K63, F70, I76, Y78, R81, W88

Heparin[2]: K34, K54, R56 + 1st mode: K20, K41, R84; 2nd mode: K72

Chondroitin sulfate[3]: 1st mode similar to HA; 2nd mode: K20, K34, K41, K54

Questions:

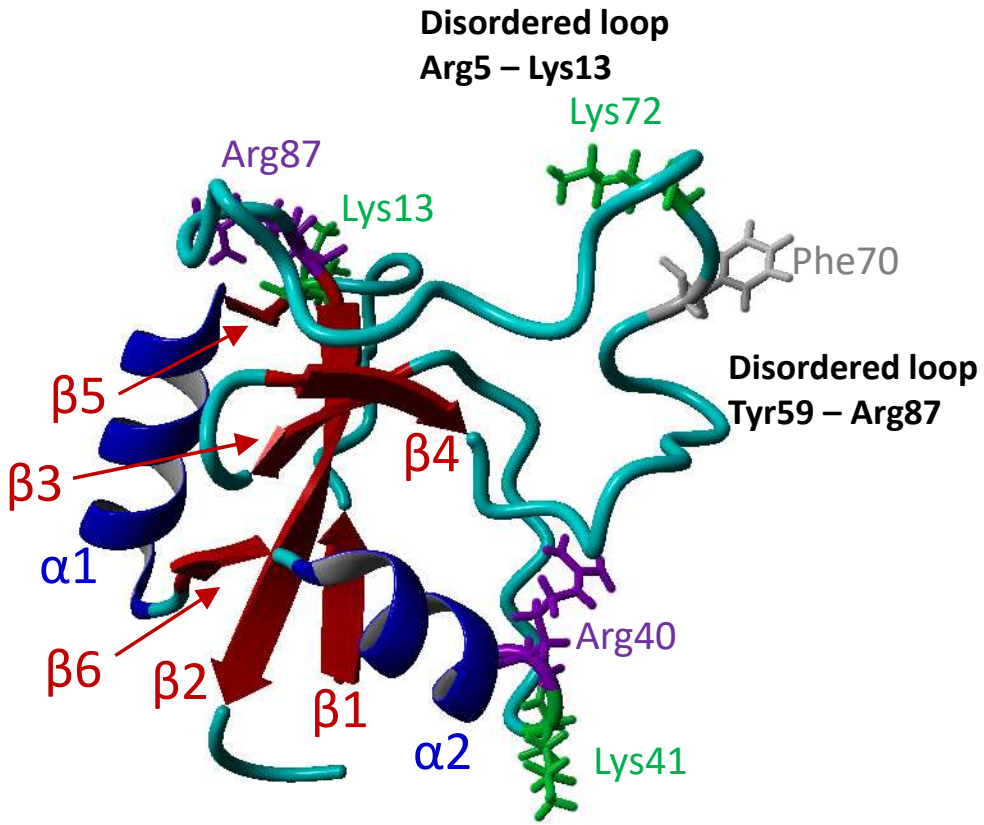
1. Are the binding sites unique and specific for individual GAGs?
2. How does electrolyte (salt) concentration influence the binding?
3. Can TSG-6 Link domain bind neutral oligosaccharides?
4. Is it possible to design artificial oligosaccharides binding hyaladherins?

Computational details:

- MD simulations carried out in GROMACS v. 5.1.1
- CHARMM 36 force field
- TIP3P model of water
- Water box 8×8×8 nm
- HA (GlcHA) oligosaccharides of 12 monosacharide units
- TSG-6 Link domain – PDB code: 2N40
- NVT ensemble
- T = 300 K
- Solvent
  - pure water
  - 0.15M NaCl

[1]Blundell et al. J. Biol. Chem. **278**, 49261 (2003)
[2]Mahoney et al. J. Biol. Chem. **280**, 27044 (2005)
[3]Park et al. Biochemistry **55**, 262 (2016)

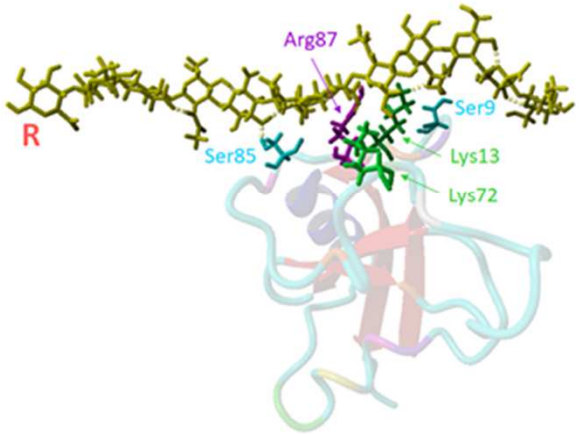
Structure of the TSG-6 Link domain

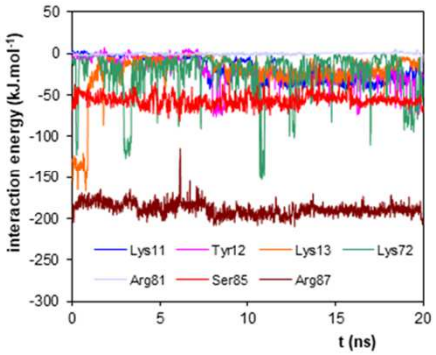


Searching for hyaluronan (HA) binding sites on TSG-6 link domain in **pure water**

Binding site 1 (BS1)

- search started from the published NMR structure
- partially corresponds to the NMR-detected site

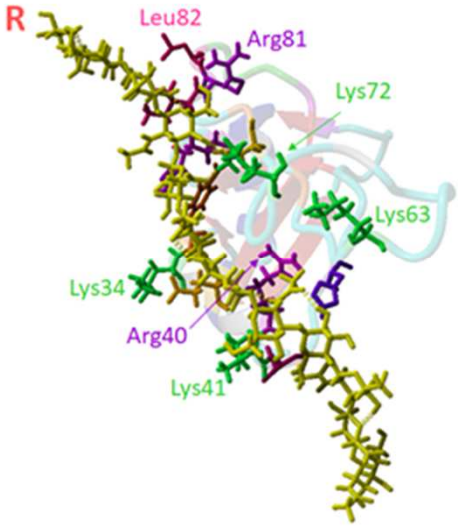


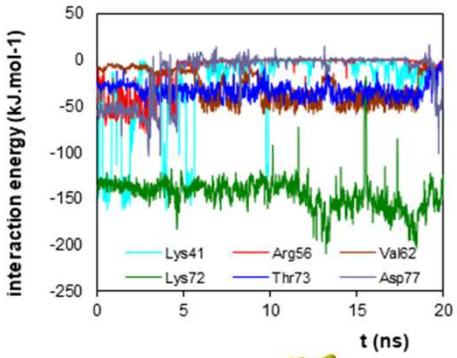


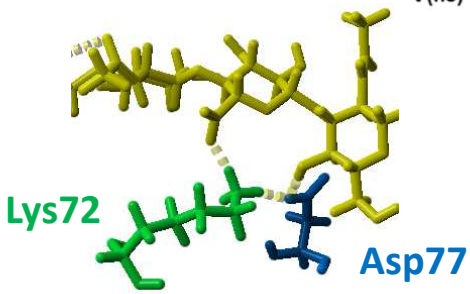
Molecular dynamics simulaton of TSG-6 link domain with hyaluronan oligosaccharide

Binding site 2 (BS2)

- found by a random simulation of HA oligosaccharide with TSG-6 Link domain
- resembles the heparin and chondroitin sulfate binding sites



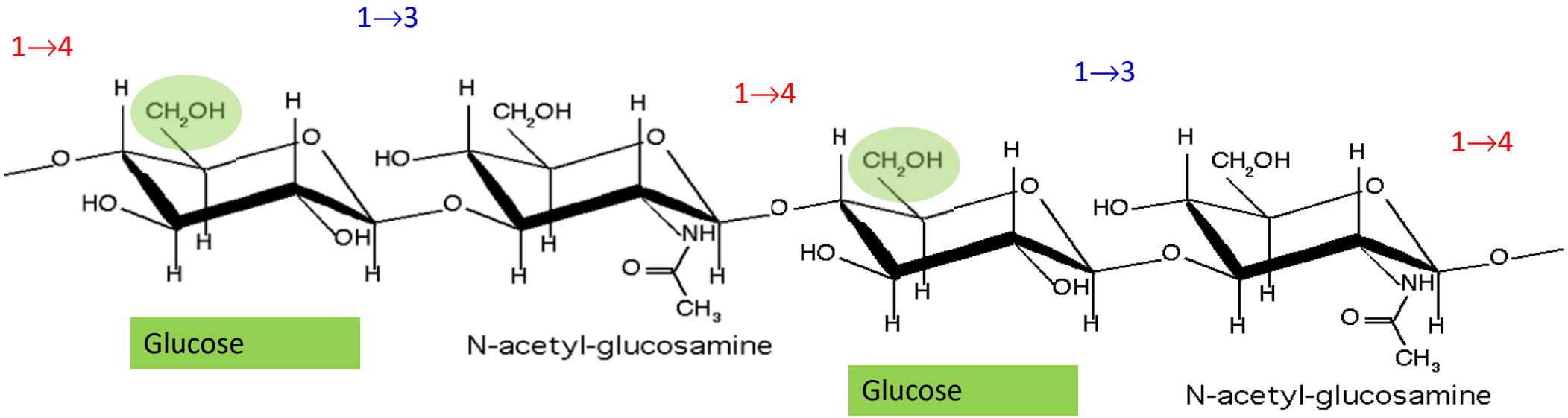




Interactions of HA and TSG-6 link domain contain a significant contribution of neutral amino acids, e.g. Ser85, Val62, Thr73, .etc.

Can TSG-6 Link domain bind also neutral oligosaccharides?

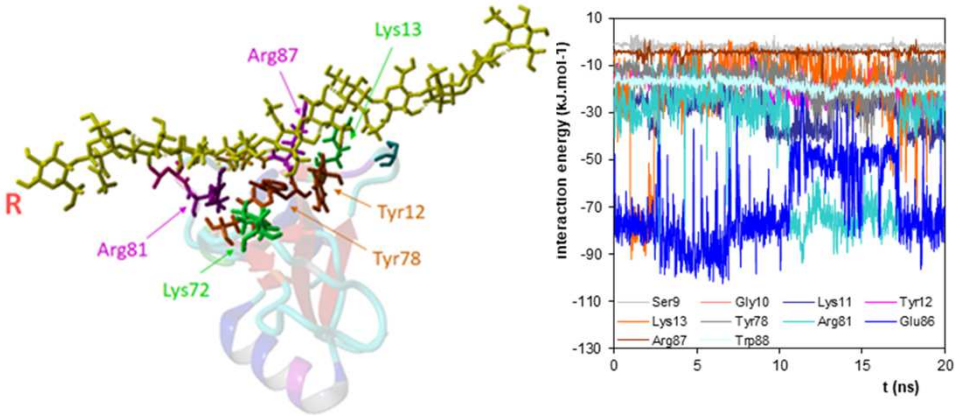
Neutral analog of hyaluronan – glucuronic acid substituted by glucose - GlcHA



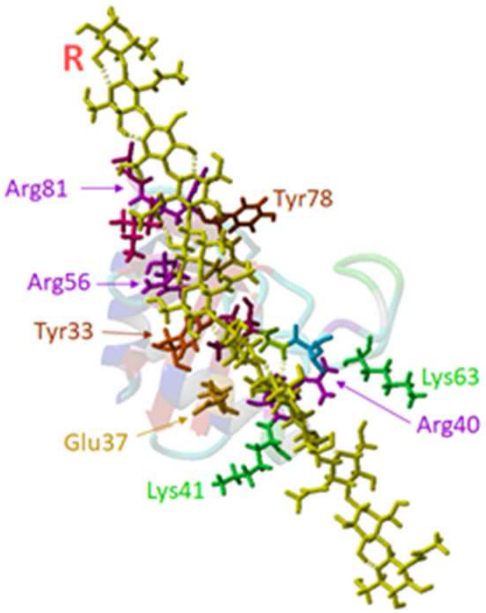
Neutral HA analog (GlcHA)

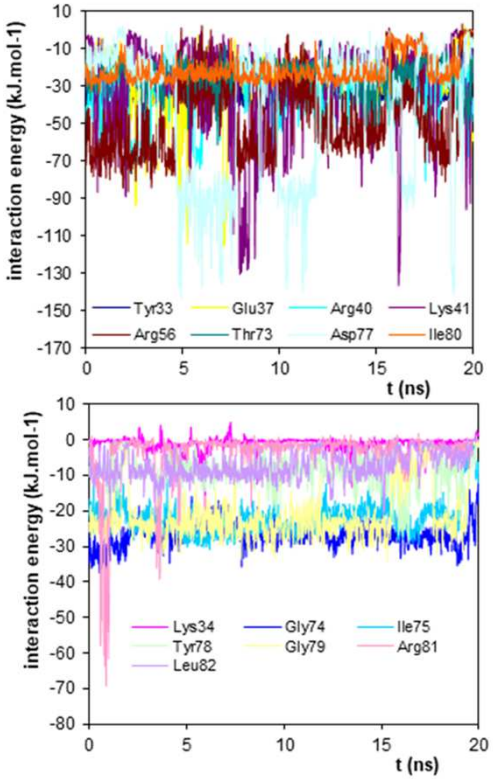
pure water

Binding site 1 (BS1)



Binding site 2 (BS2)





Geometry of both the binding modes is similar to HA.

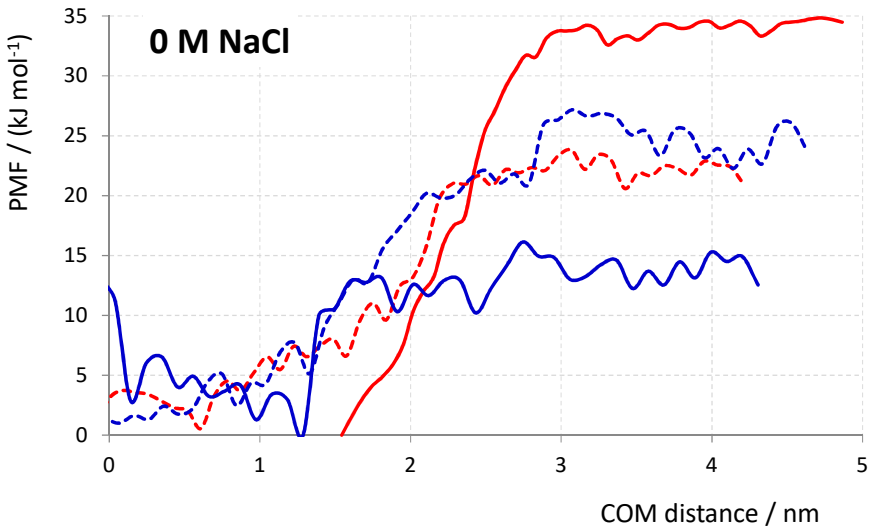
What about the binding stability?

Stability of hyaluronan binding

➡ Umbrella sampling calculations of hyaluronan-TSG-6-Link complexes

1. Pulling (SMD) run of the oligosaccharide from the protein for 10 ns, velocity 0,5 pm/ps, force constant 1000 kJ mol^{-1} nm^{-2}
2. Approx 40 configurations from the SMD run were chosen and biased equilibrium simulations under the load of an external force were carried out (10 ns each)
3. Potential of mean force calculation using the weighted histogram analysis method (WHAM)

Helmholtz free energy of the oligosaccharide binding



HA–BS1: $K_d = 8.0\times10^{-7}$ ⟺ **Experimental value for HA octasaccharides: $K_d = 1.7\times10^{-7}$**

Blundell et al. (2003) J. Biol. Chem. 278 (49), 49261.

GlcHA–BS2: $K_d = 4.4\times10^{-4}$

HA–BS2: $K_d = 1.2\times10^{-4}$

GlcHA–BS1: $K_d = 3.7\times10^{-3}$

- Hyaluronan oligosaccharides show bind to both the binding sites
- Binding in BS1 is remarkably more stable than in BS2 – likely for this reason BS2 was not detected by NMR

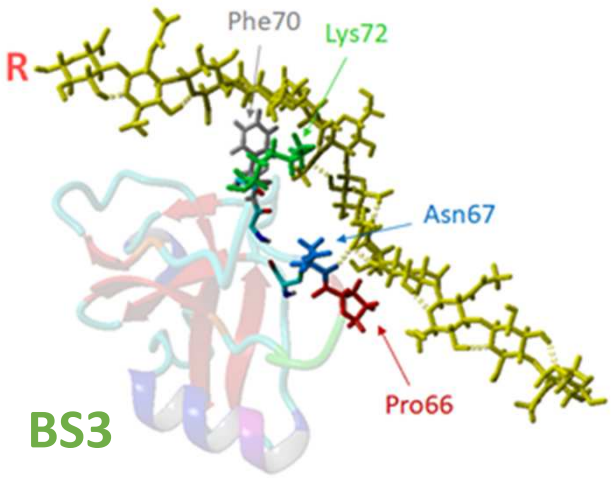
- GlcHA oligosaccharides bind to both the binding sites, too, but their stability is reversed
- Stability of GlcHA binding in BS1 is vry weak

TSG-6 Link domain can bind both charged and neutral oligosaccharides!

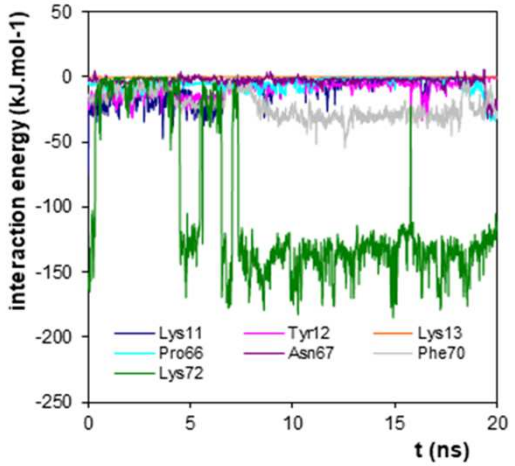
Pure water is a non-physiological environment.

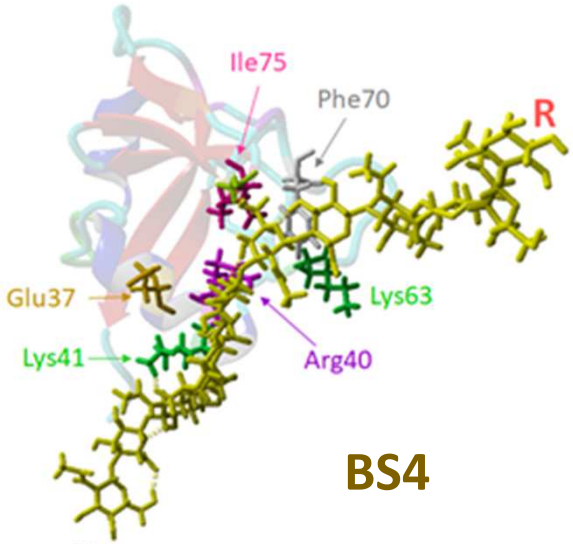
Let’s do the simulations in physiological solution (0.15M NaCl)!

HA

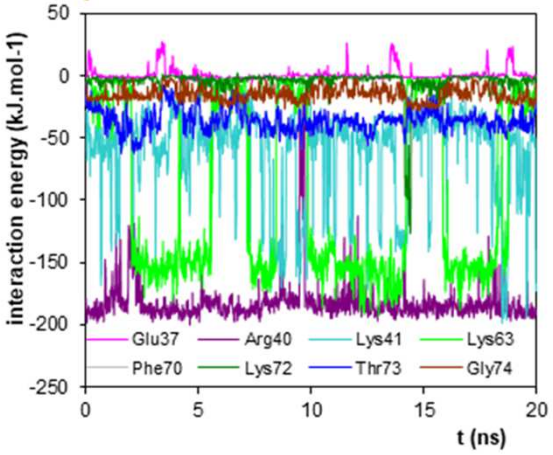


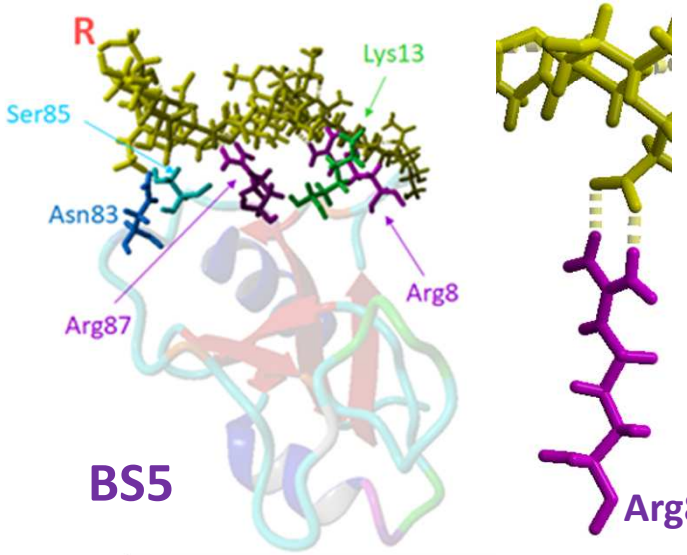
BS3



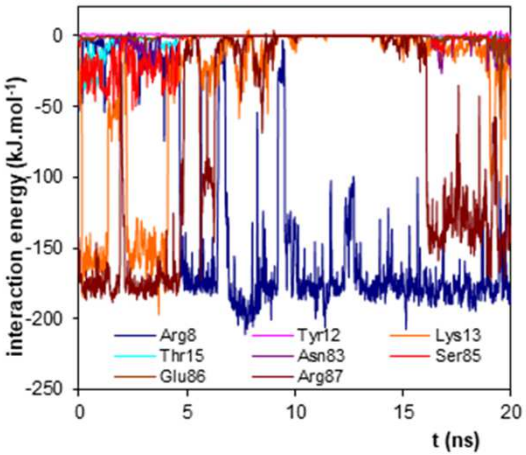


BS4

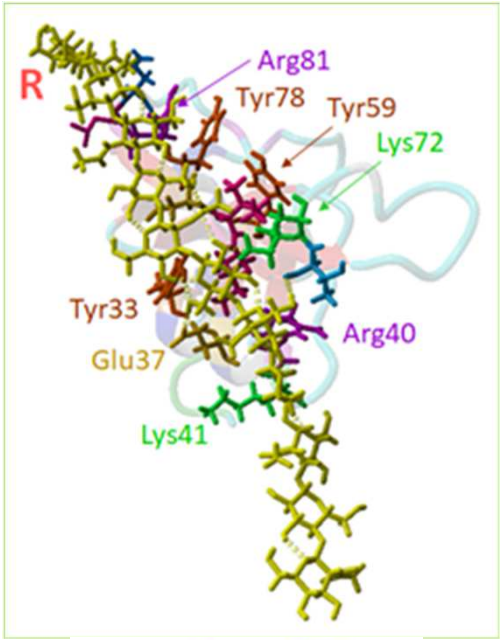


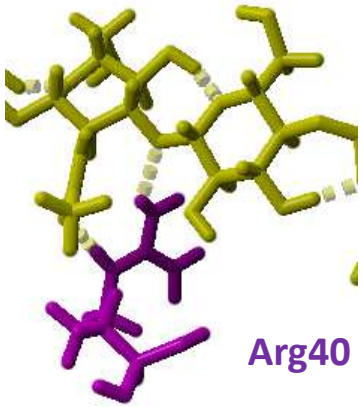


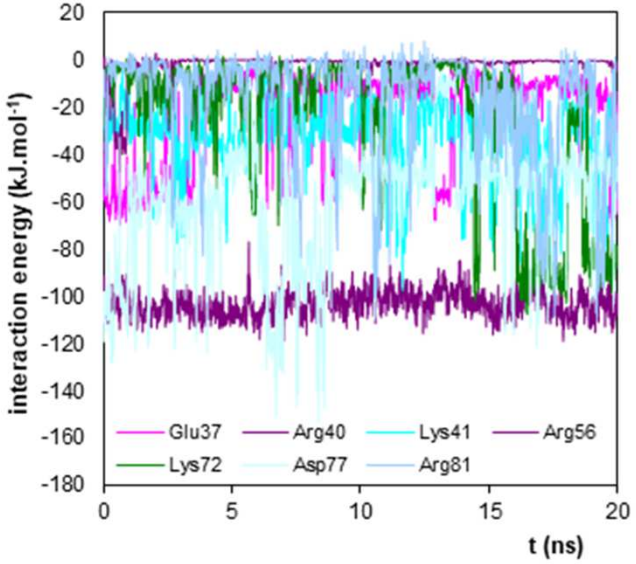
BS5

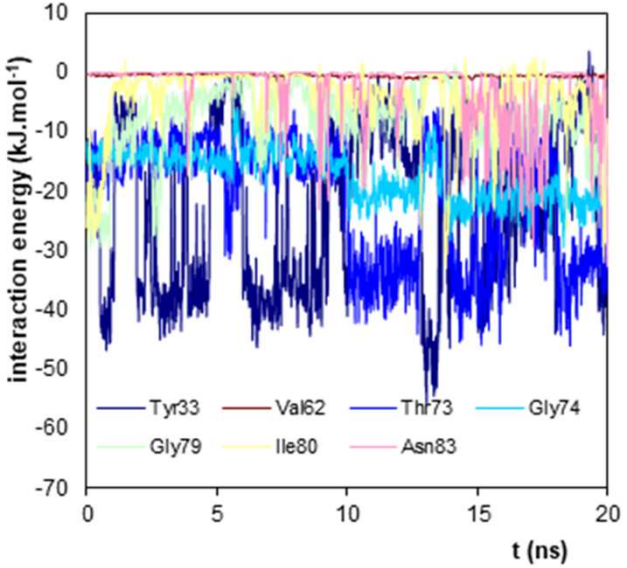


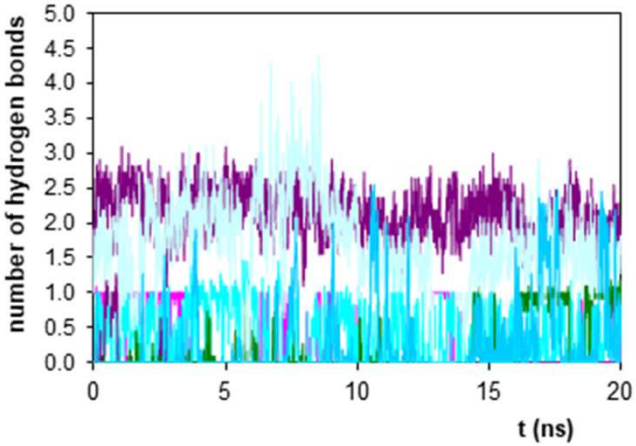
GlcHA

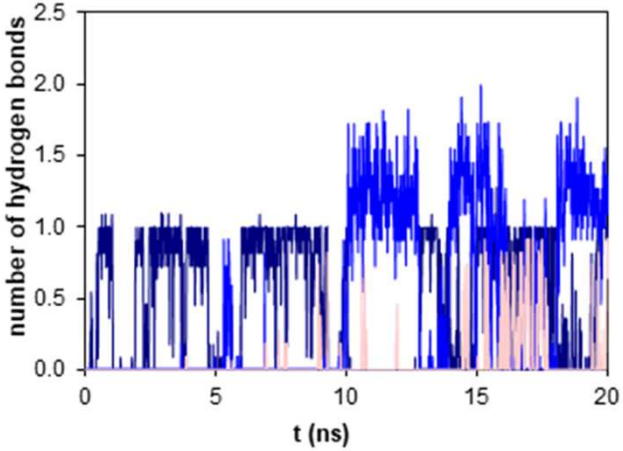






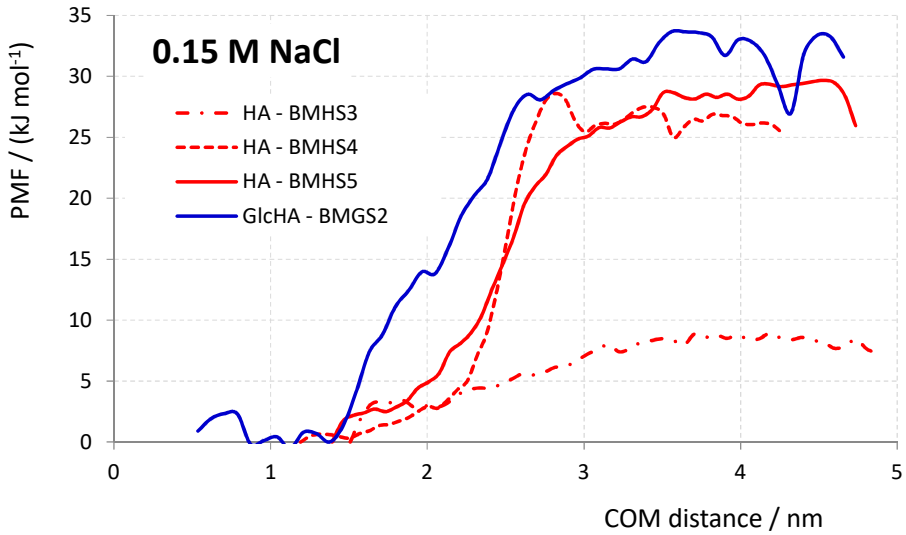






Interaction energies follow the number of hydrogen bonds between amino acids and the ligand.
(Valid generally for all the modes.)

Stability of the binding modes in 0.15M NaCl



GlcHA–BS2: $K_d = 1.8\times10^{-6}$

HA–BS5: $K_d = 1.3\times10^{-5}$

HA–BS4: $K_d = 3.0\times10^{-5}$

HA–BS3: $K_d = 4.0\times10^{-2}$

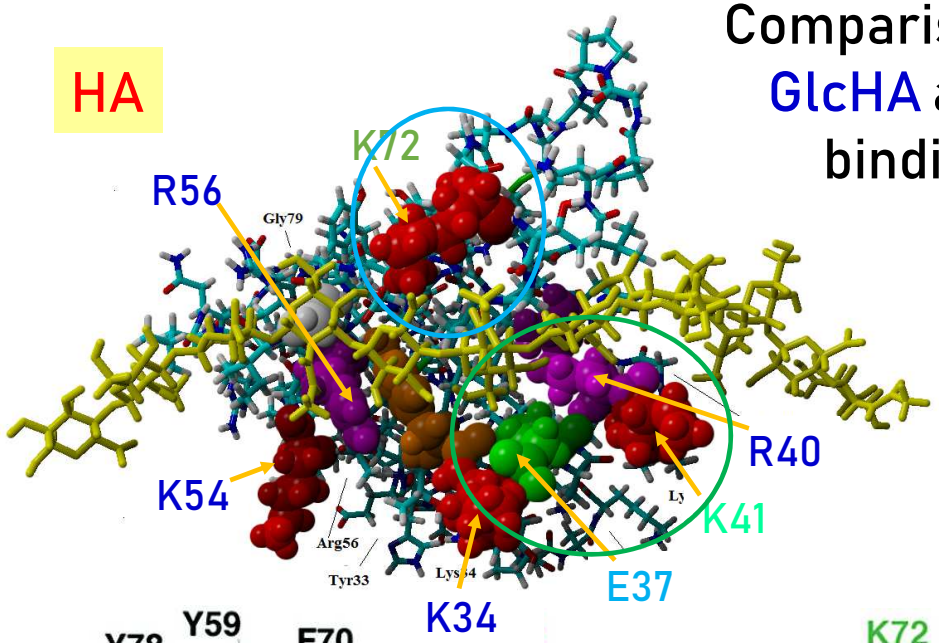
- Two stable HA binding modes were found – BS4 and BS5
- Both these modes are less stable than in pure water
- Binding mode in BS3 is rather unstable

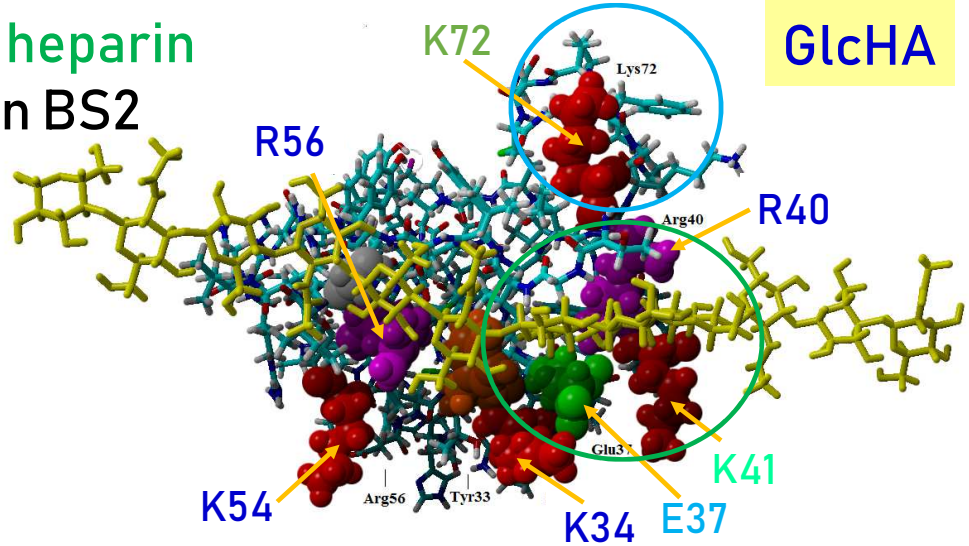
- GlcHA binds to the binding site BS2, geometrically almost equal to BS2 in pure water
- The stability of this binding mode is considerably higher than in pure water

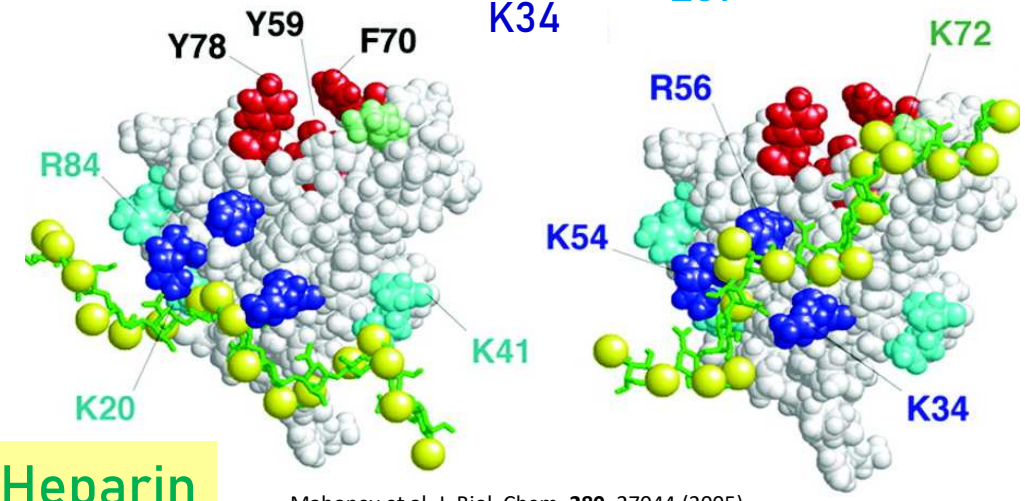
In 0.15M NaCl:

- HA binding modes are weaker due to the screening of the electrostatic interaction
- GlcHA modes are more stable due to the stronger hydrophobic interaction between the neutral amino acids and the ligand

Comparison of HA and GlcHA and heparin binding in BS2





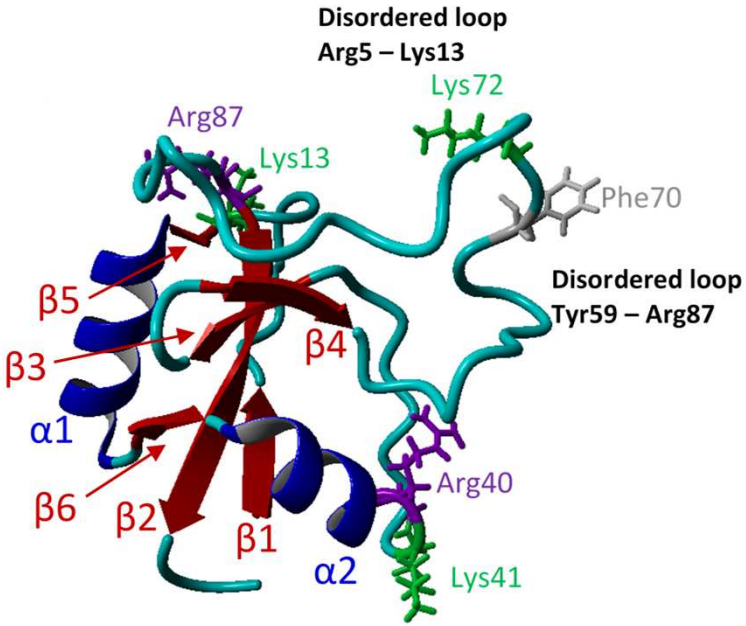


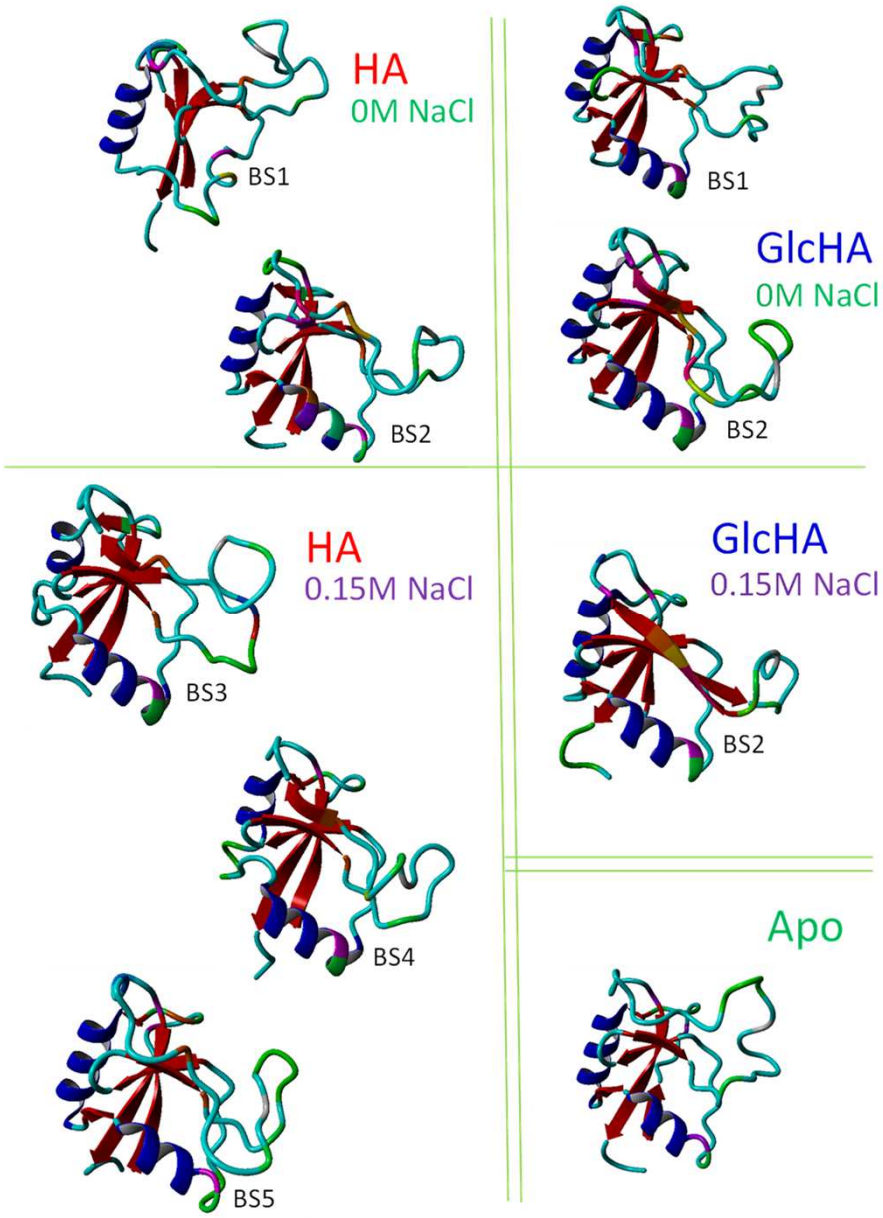
Mahoney et al. J. Biol. Chem. **280**, 27044 (2005)

- BS2 is a „combination“ of the two heparin binding modes detected by NMR
- It involves the amino acid residues of both the modes after a moderate conformation change
- Both oligosaccharides are bound in a similar way

Protein flexibility stabilizes the protein-ligand complexes

- the shape of the protein molecule can adapt to the ligand enabling the formation of more hydrogen bonds and other interactions
- different binding modes show different 3D structure





Conclusions

Thanks to …

- Roman Witasek (PhD student)
- Eva Kutálková
- Josef Hrnčiřík

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