



Inhibition Of Mammalian Glycoprotein YKL-40 : Identification Of Potential Physiological Ligand

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Introduction to YKL-40

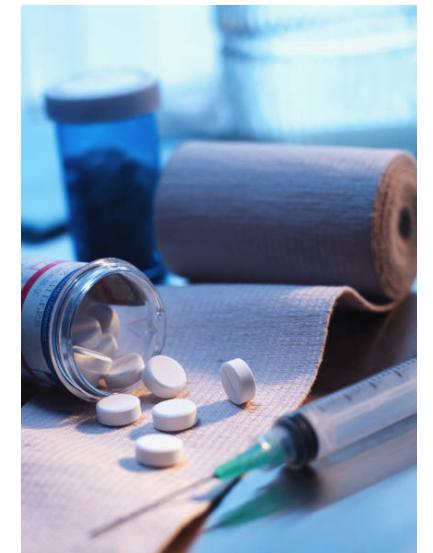
- Categorized as non-catalytic mammalian glycoprotein

Glycoprotein	Disaccharide of GlcNAc covalently bound at N60
Mammalian	<i>In vivo</i> secretion by synovial cells, chondrocytes, endothelial and epithelial cells, and tumor cells in mammals.
Non-catalytic	<ul style="list-style-type: none">• Homologous to Glycoside Hydrolase (GH) Family 18 chitinases• Substitutions in motif essential for catalysis $DXXDXDXE \rightarrow DXXDXAXL$• Lectin – a non-catalytic sugar binding protein

- Also known as CHI3LI (chitinase 3-like 1), HCGP-39 (human cartilage glycoprotein-39)

YKL-40: Biological Function?

- **Significance as Biomarker** – high expression levels associated with chronic inflammatory diseases, multitudes of cancers and more.
- **Therapeutic Target** – promotes tumor angiogenesis and involves in tissue remodeling – potential therapeutic target in several disorders.



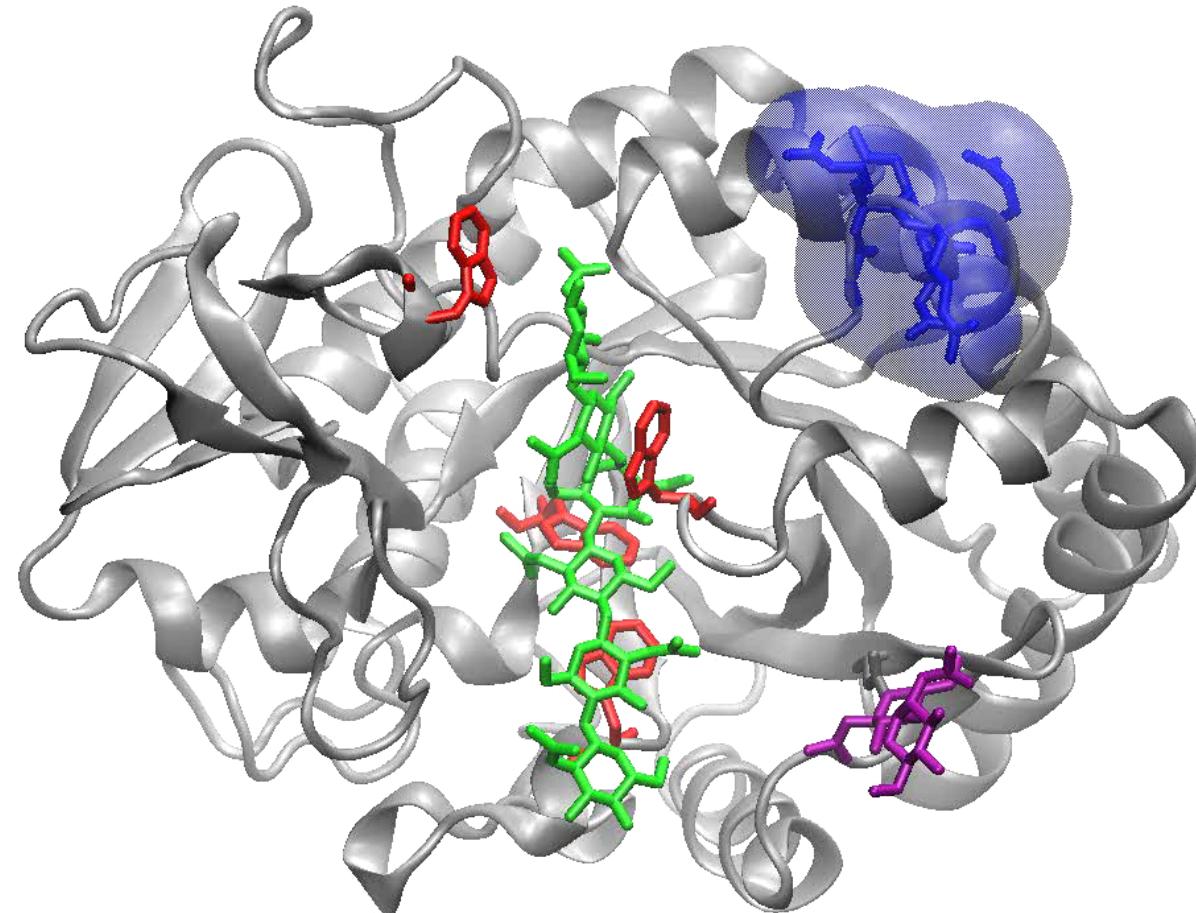
Johansen J.S., Dan. Med. Bull., (2006)

Binding Sites of YKL-40

1

2

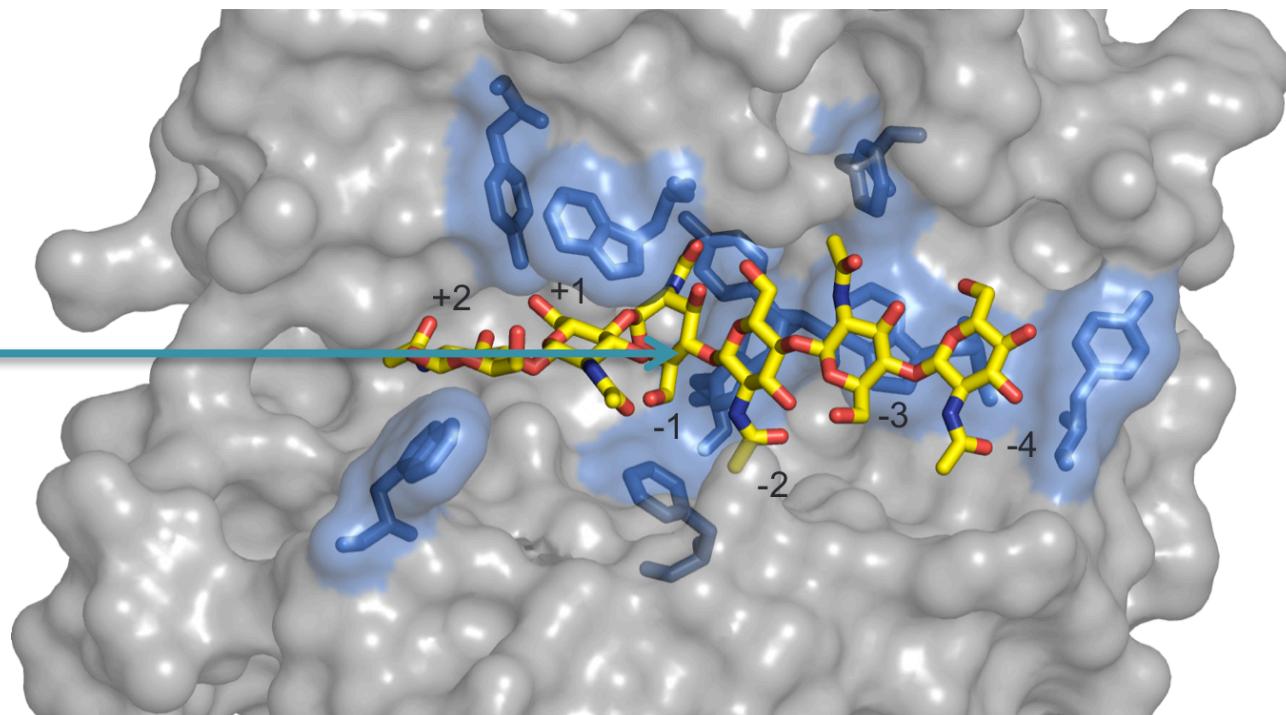
More ?



Chitin-bound YKL-40 structure by Houston et al., J. Bio. Chem., (2003)
PDB ID : 1HJW

Conventional Carbohydrate Binding Site

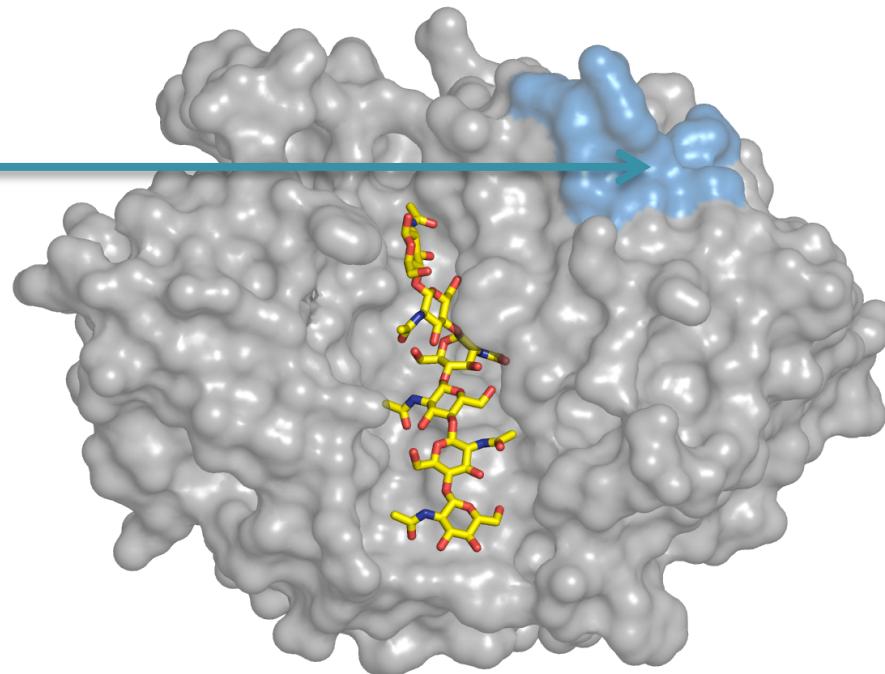
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- YKL-40 has been shown to bind chito-oligosaccharides through X-ray crystallographic study
- **Chitin** – polysaccharide of N-acetyl glucosamine (GlcNAc)
- Binding cleft with 9 sugar-binding subsites from +3 to -6
- Potential binding site for similar carbohydrates?

2

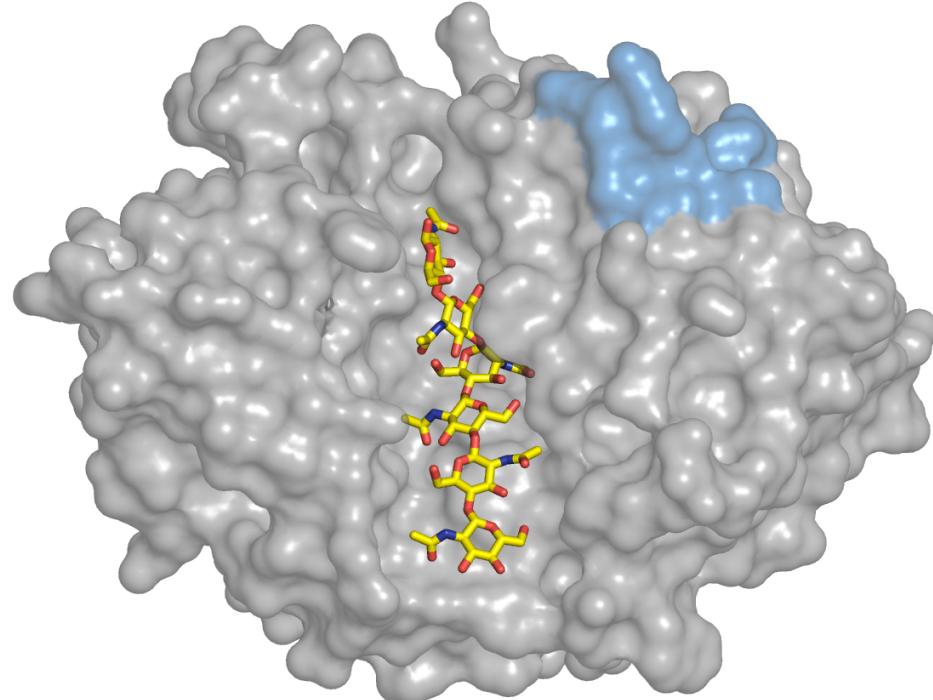
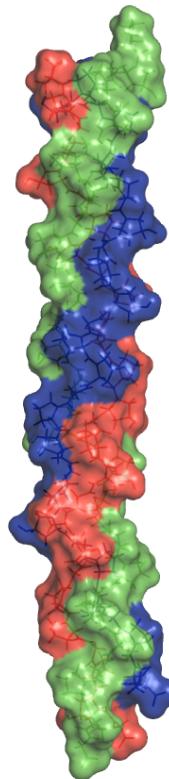
Putative Surface Binding Site for Heparin



- Affinity for heparin aids in purification of YKL-40.
- **Heparin** – Highly sulfated carbohydrate found in extracellular matrix (ECM).
- No structural evidence of heparin bound at the binding site.
- Complementary features of heparin and surface binding site?

More ?

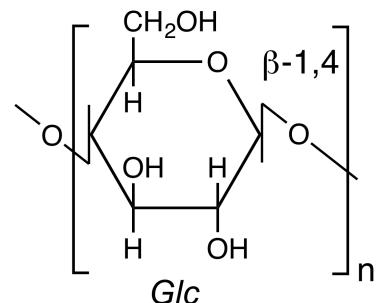
Protein-protein Interaction of YKL-40



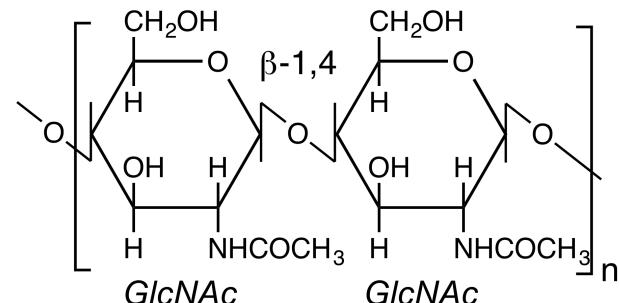
- Specific binding affinity for three types of collagen
- **Collagen** – triple helical protein also comprises most of ECM
- Ambiguous effects of YKL-40 binding on fibril formation of collagen
- **No binding site characterization** - no structural data for this protein-protein complex

Potential Carbohydrate Ligands

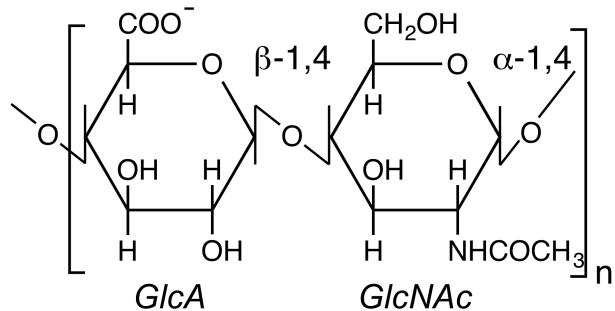
Cello-oligomer



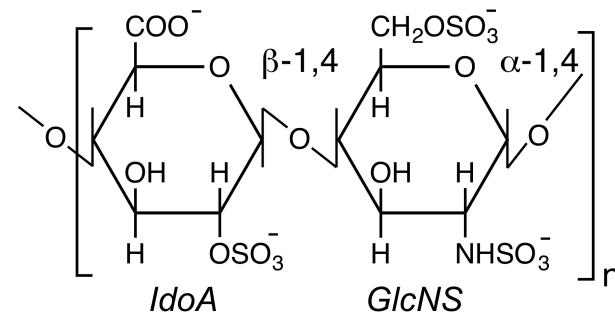
Chitin



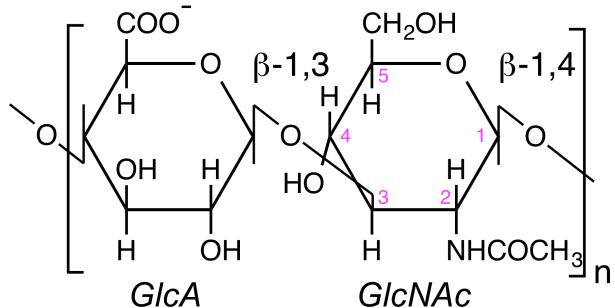
Heparan Sulfate



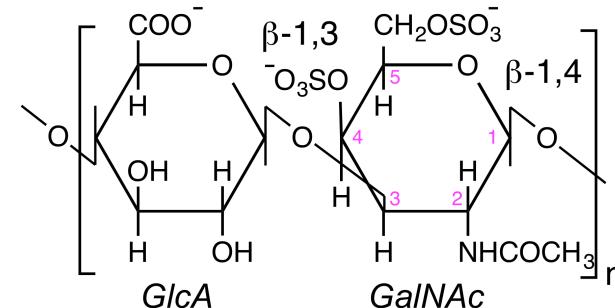
Heparin

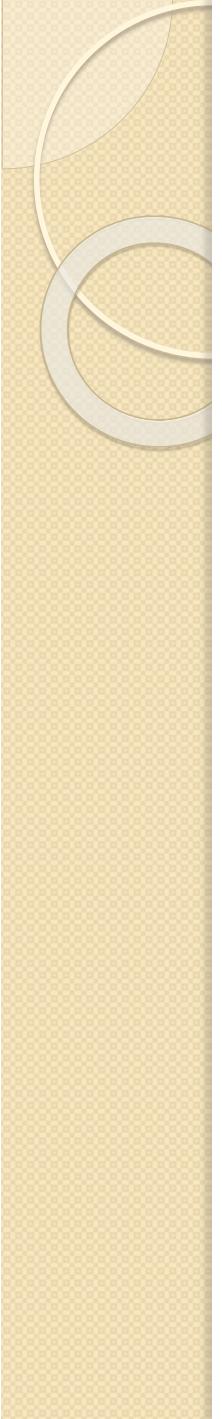


Hyaluronan



Chondroitin Sulfate

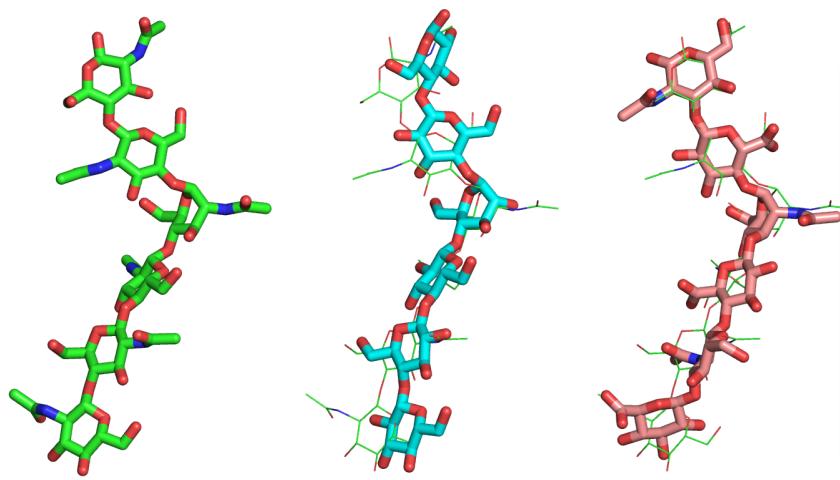




Computational Approach

1. Docking of ligand in the binding cleft and setup the protein-ligand complex.
2. Solvation and energy minimization of protein-ligand-solvent system.
3. Heating and equilibration of the system for 100 ps using CHARMM.
4. Production run of molecular dynamic simulation for 250 ns in canonical ensemble using NAMD.
5. Binding free energy calculation by FEP/ λ -REMD.
6. Analysis of the trajectories and comparison of ΔG for all ligands.

Over the 250 ns simulation



Chitin

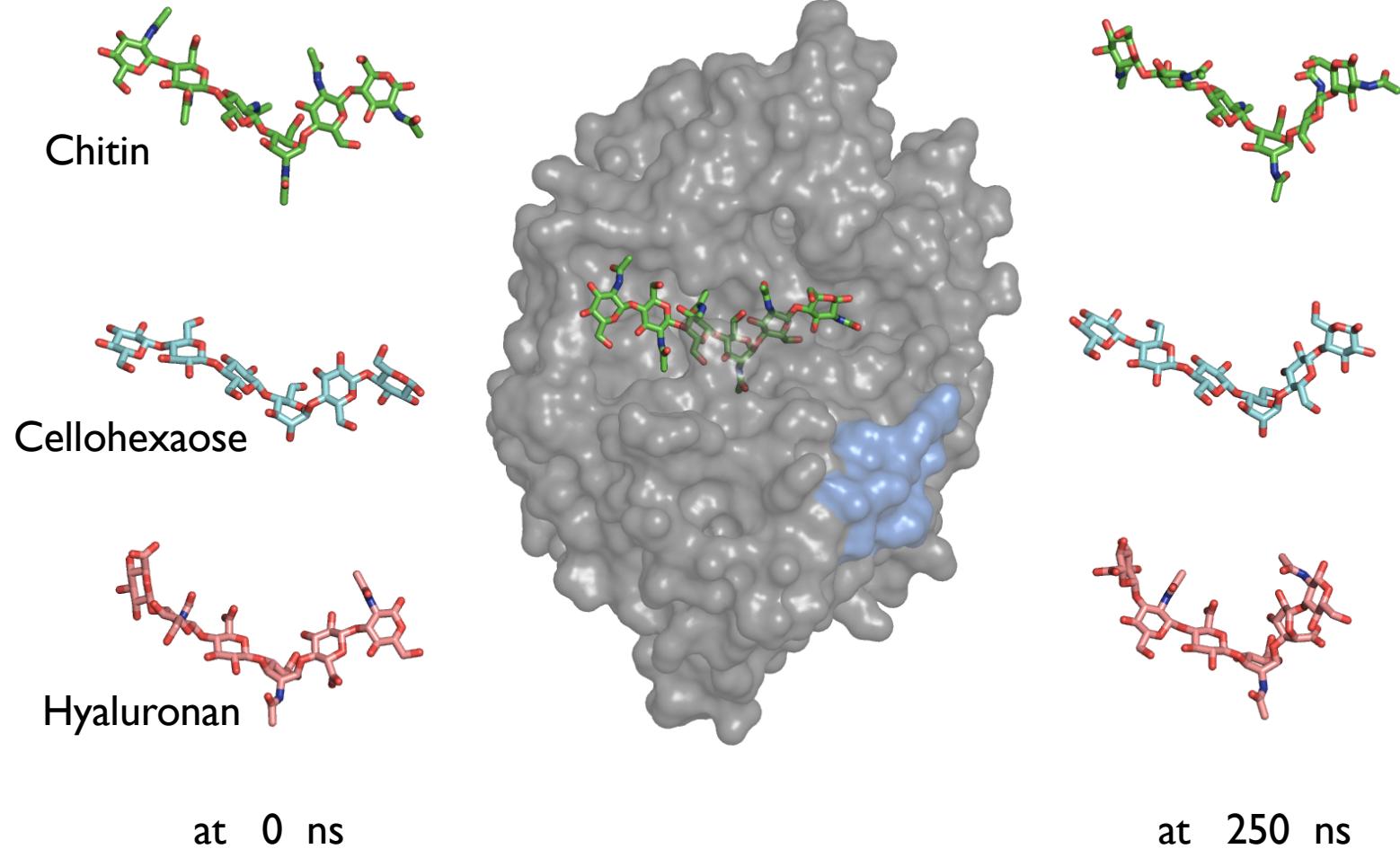
Cellohexaose

Hyaluronan



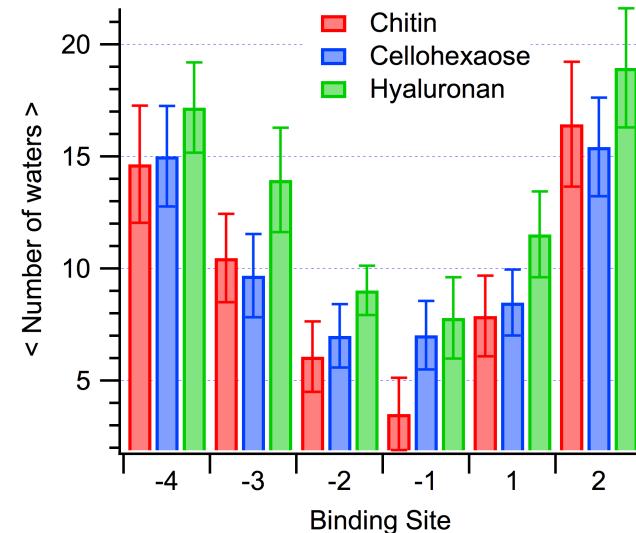
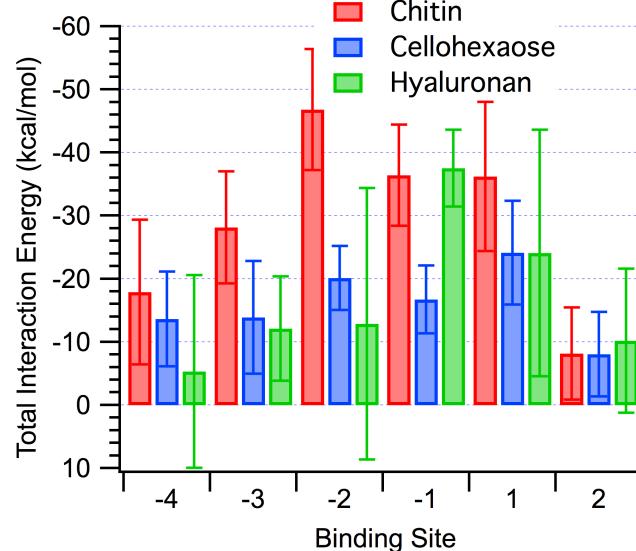
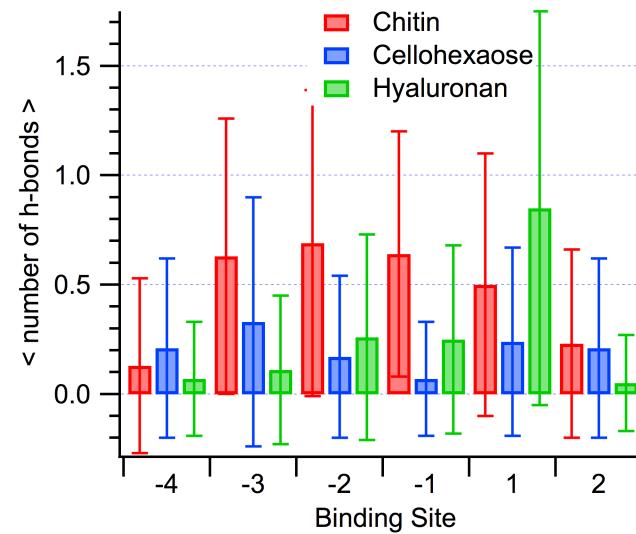
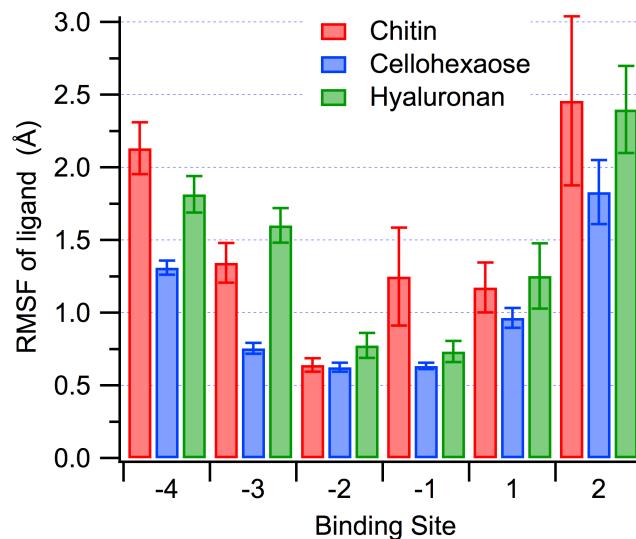
Exit the binding cleft

Who Is Welcome In The Cleft?



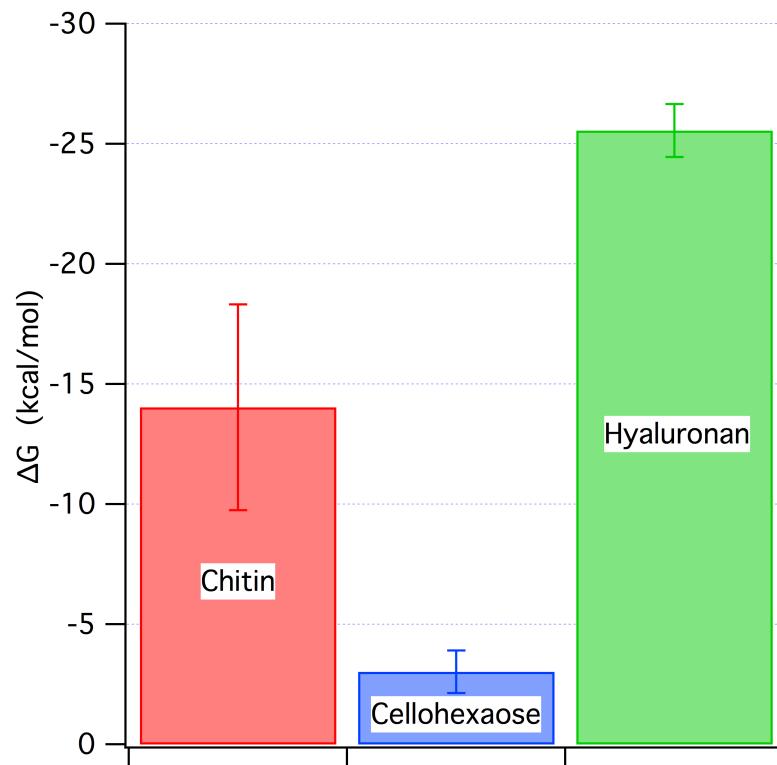
Hyaluronan : Potential Physiological Ligand ?

Analysis of polysaccharide binding dynamics



Hyaluronan : Potential Physiological Ligand ?

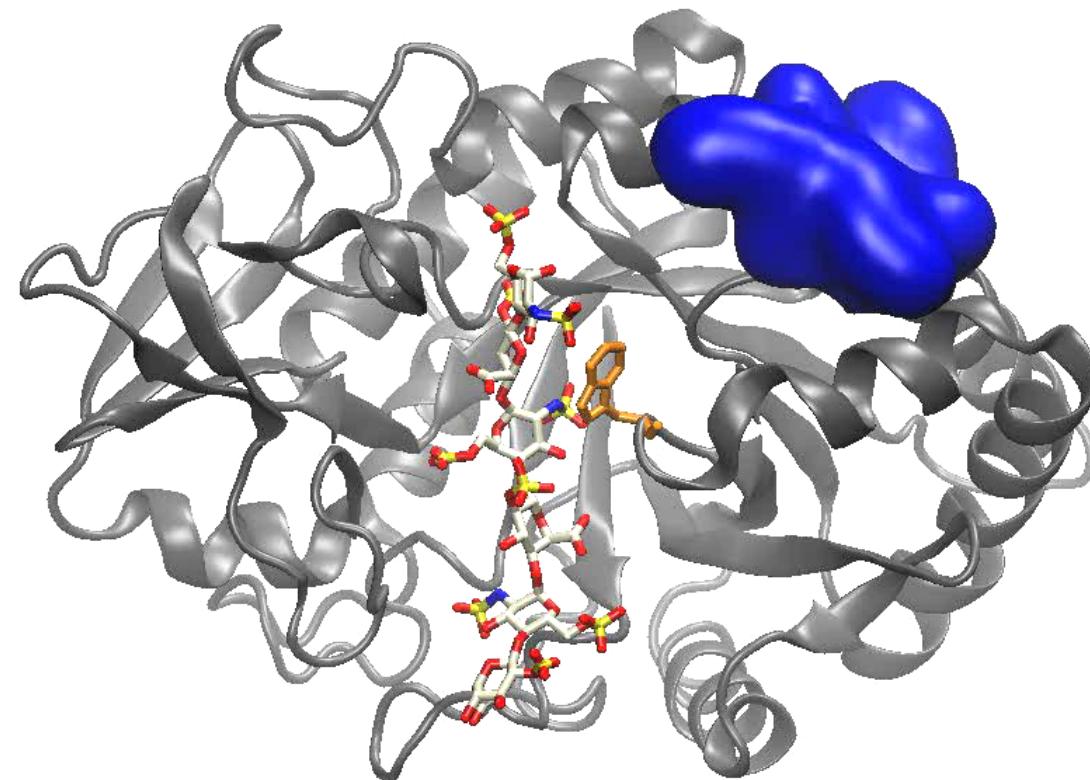
Relative affinity in terms of absolute binding free energy



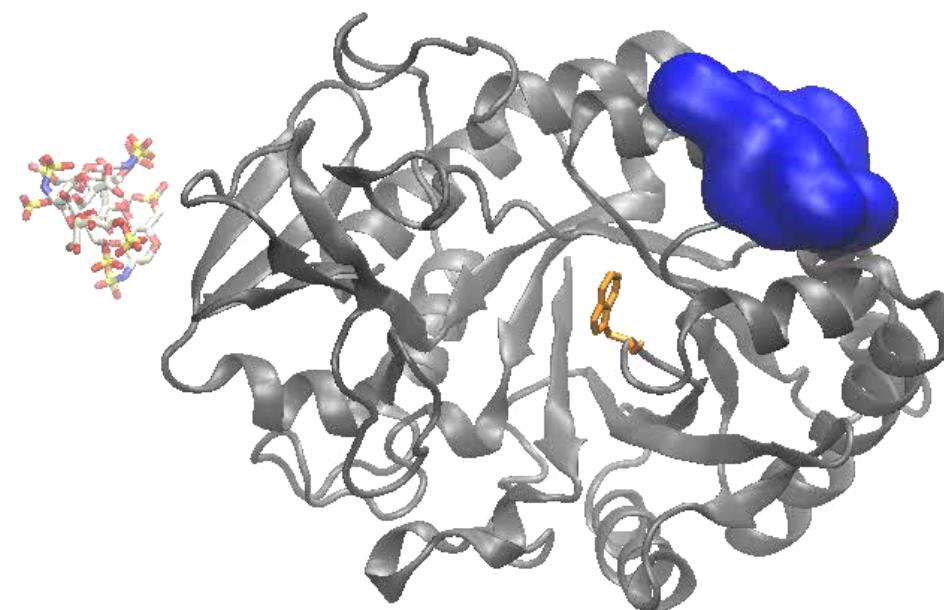
Absolute binding free energy of chitin, glucose and hyaluronan to YKL-40 calculated by FEP/ λ -REMD method.

- Cellohexaose out of race due to low potential for enthalpic contribution.
- YKL-40 binds chitin with similar affinity as other GH Family 18 chitinases. (Humre AG, Jana S et. al. - Submitted)
- Hyaluronan exhibits enthalpic contributions similar to chitin, which is likely related to hydrogen bond formation.
- Negative charge on hyaluronan significantly contributes to the electrostatic interactions, accounting for the difference between chitin and hyaluronan.

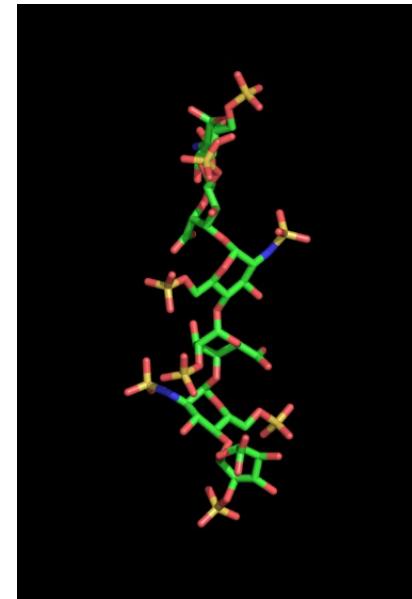
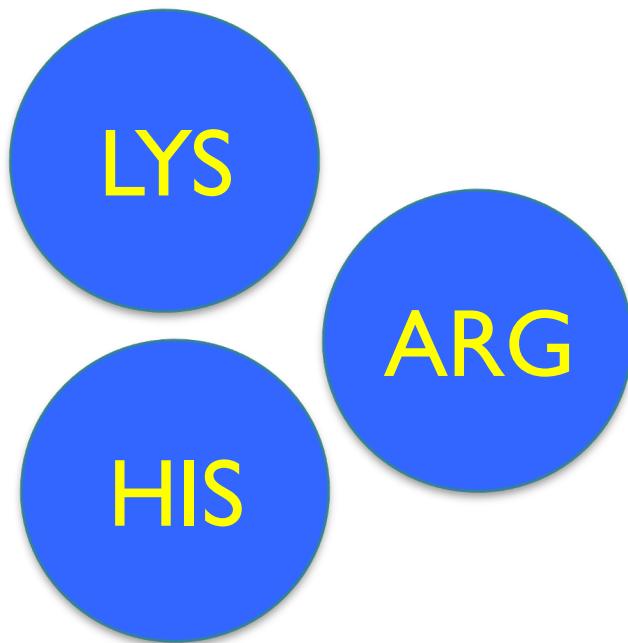
What Happens To Heparin?



Starting From Different Coordinates!



What Amino Acids Comprise This Site?



Solution
structure
of
Heparin

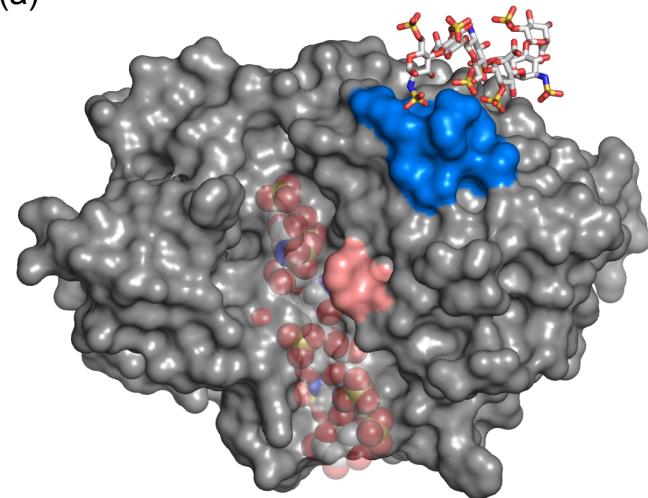
- Comparison with heparin-binding consensus sequences

X-B-B-X-B-X-B → G-R-R-D-K-Q-H

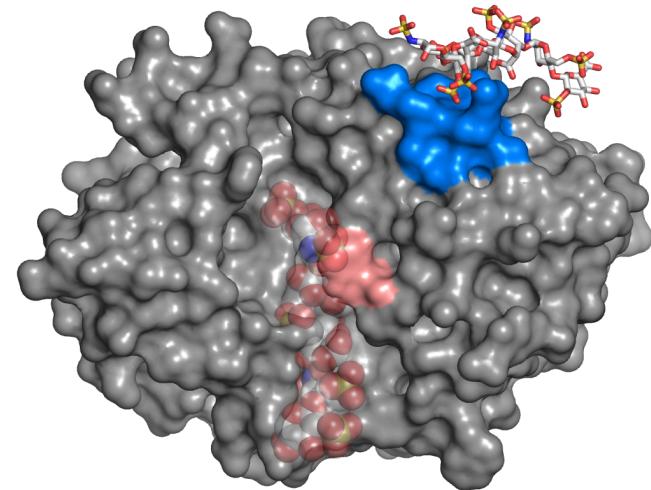
where B is basic amino acid and X is neutral or hydrophobic amino acid residue.

Heparin Binding : Specific or Non-specific ?

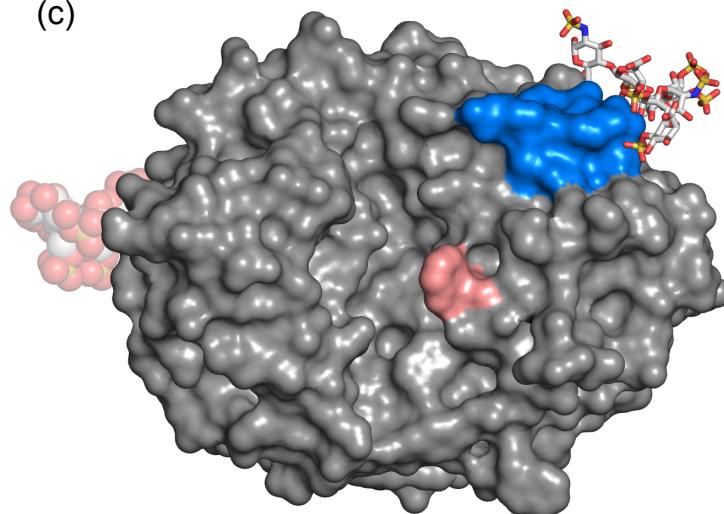
(a)



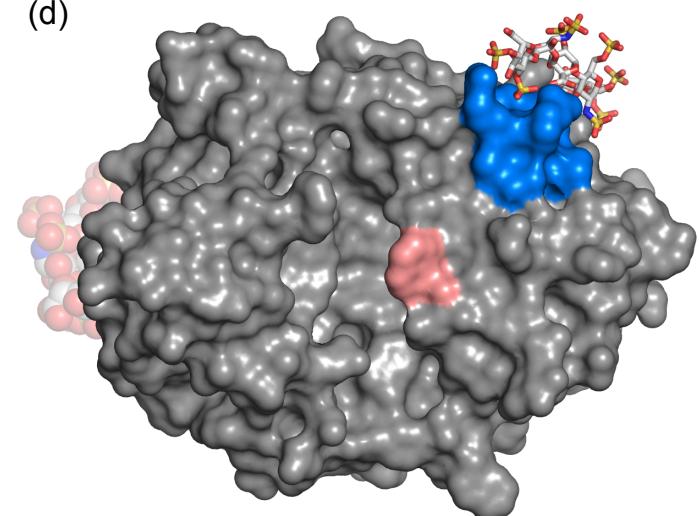
(b)



(c)



(d)



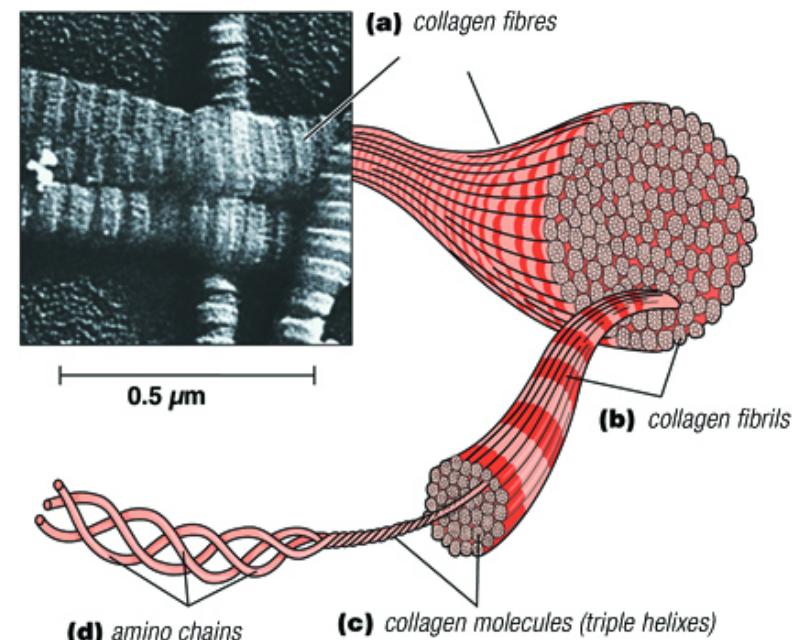
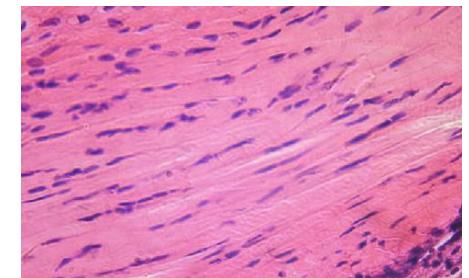
Heparin (white stick representation)

Putative heparin-binding site of YKL-40 (blue surface representation)

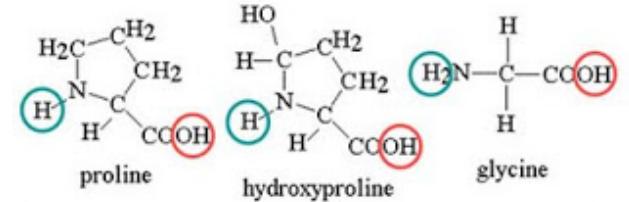
Primary binding site marked by an aromatic residue (pink surface representation)

Protein-protein Interactions of YKL-40 : Affinity For Collagen

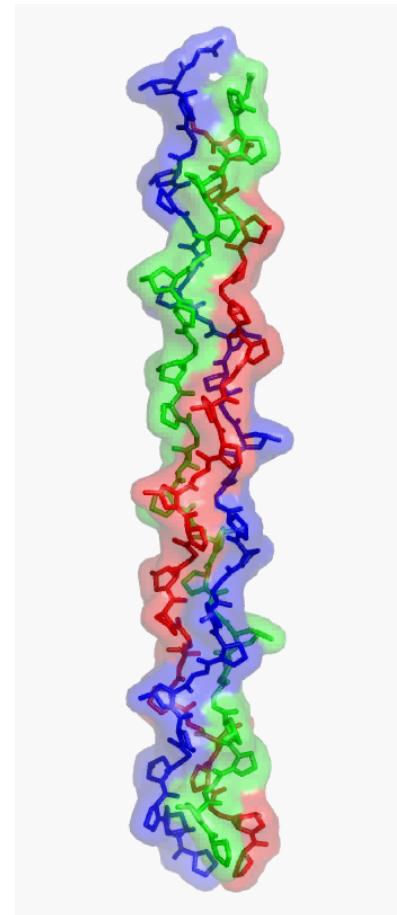
- YKL-40 is mostly expressed in connective tissue especially in cartilage.
- Collagen fibers are significant components of connective tissue accounting to almost 25% of total protein in mammalian body.
- Unique isoforms of YKL-40 extracted from different cells display ambiguous effects on collagen fibril formation.



Collagen



- Collagen is macromolecular protein with triple helical structure
- basic **Gly** – **Pro** – **Hyp** repeating amino acid sequence
- 27 different types of collagen
- 10/3 and 7/2 helical symmetries
- Four collagen peptides selected to represent helical and amino acid variability



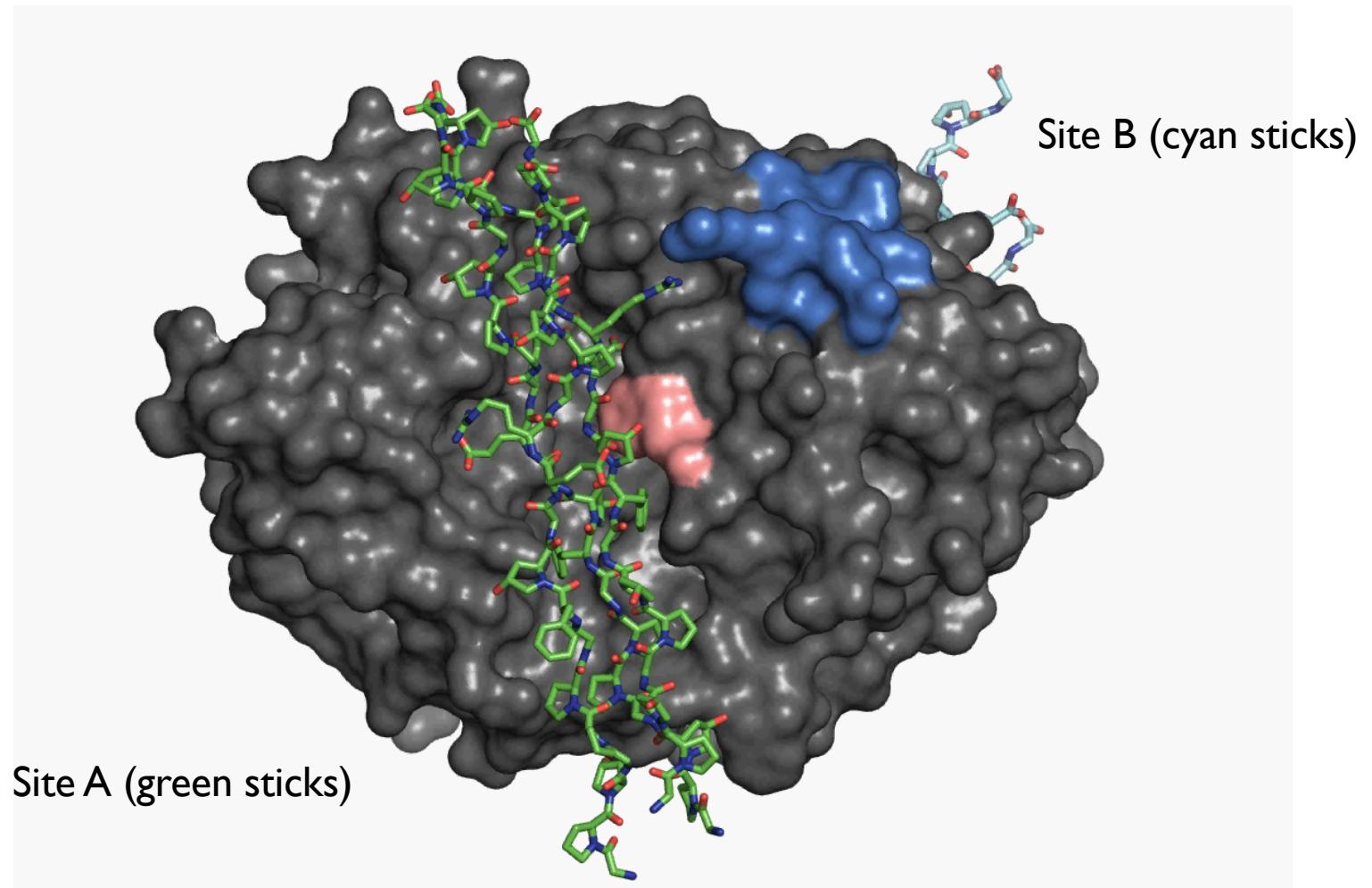
PDB ID : ICAG

Collagen: Representative Peptide Models

- ICAG
 - Basic model consisting only Gly-Pro-Hyp repeatedly, with one mutation of **Gly → Ala** and relaxed 7/2 symmetry
- ICAG_unmut
 - Same as ICAG model without mutation with perfect 7/2 symmetry.
- IBKV
 - Collagen-like peptide consisting sequence, **GITGARGGLA**, in middle from human type III collagen with 10/3 symmetry
- IQ7D
 - Collage-like peptide consisting **GFOGER** motif known to bind the integrin $\alpha 2\beta 1$ -I receptor protein with mixed symmetry

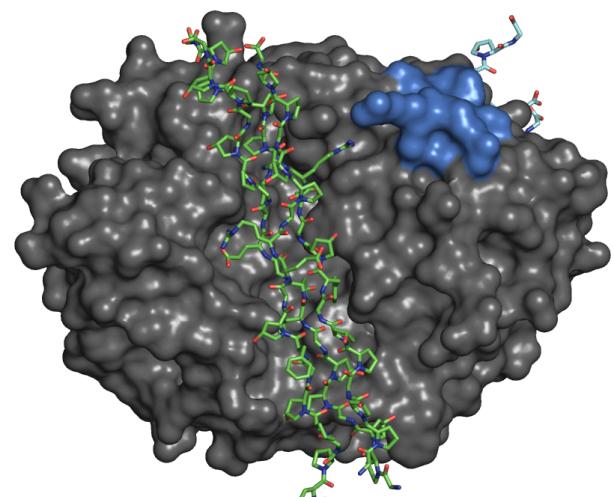
Hydroxyproline

Where Is The Collagen Binding Site?

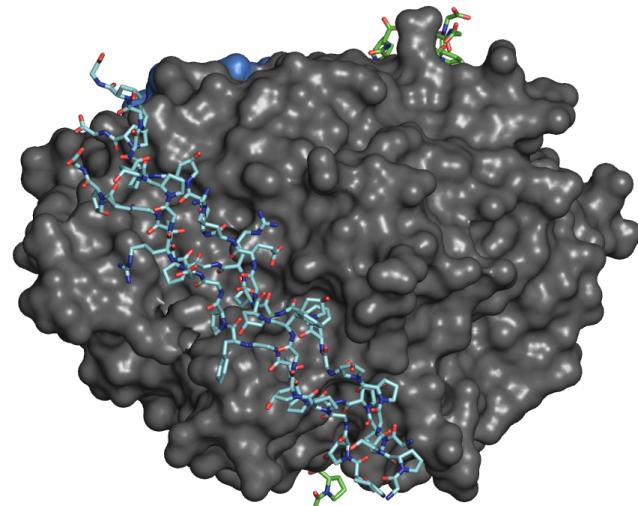


Molecular shape complementarity docking calculations predict collagen-like peptides can bind to YKL-40 in **TWO** possible orientations.

Binding Dynamics at Site A and Site B



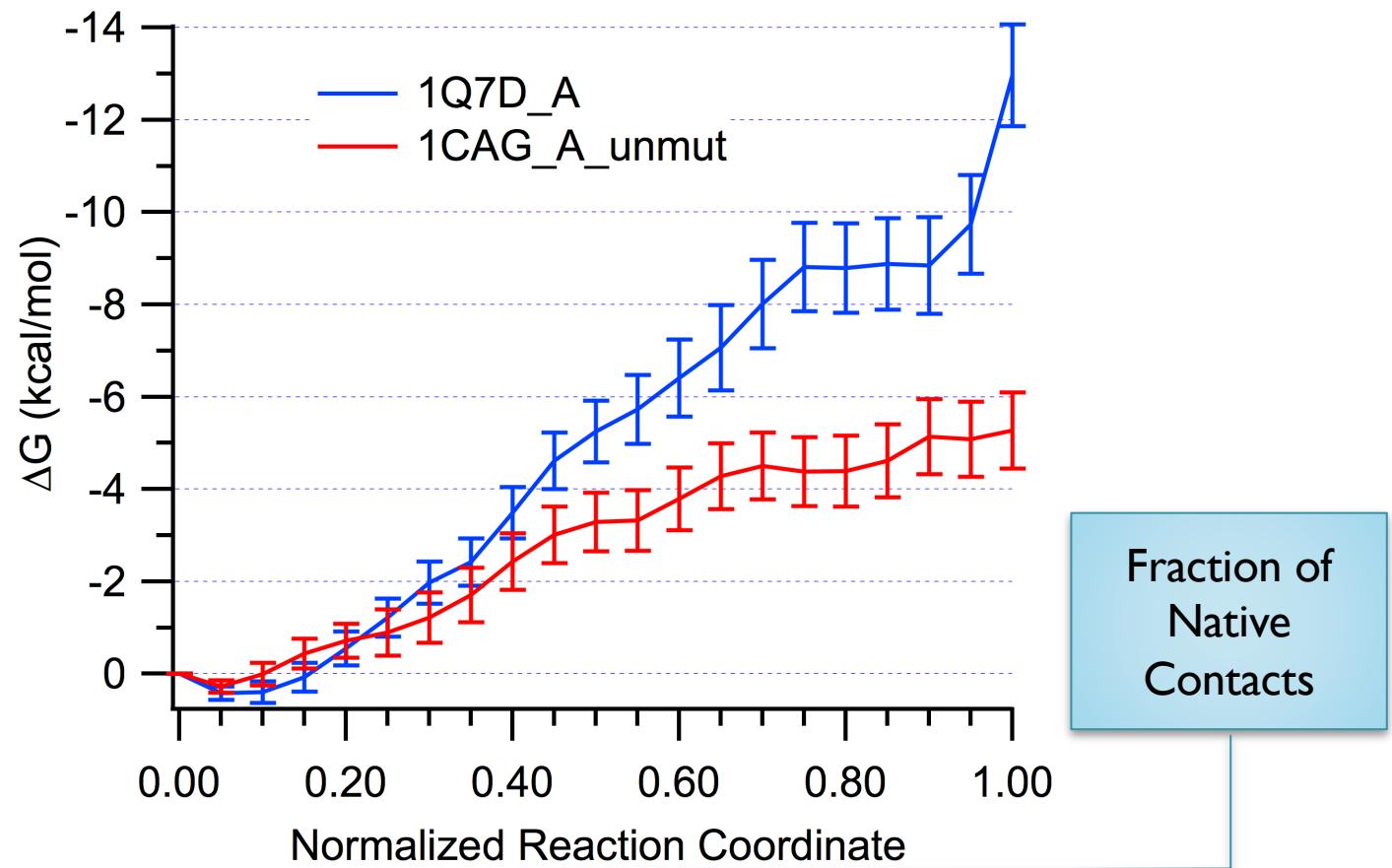
Site A (collagen in green sticks)



Site B (collagen in cyan sticks)

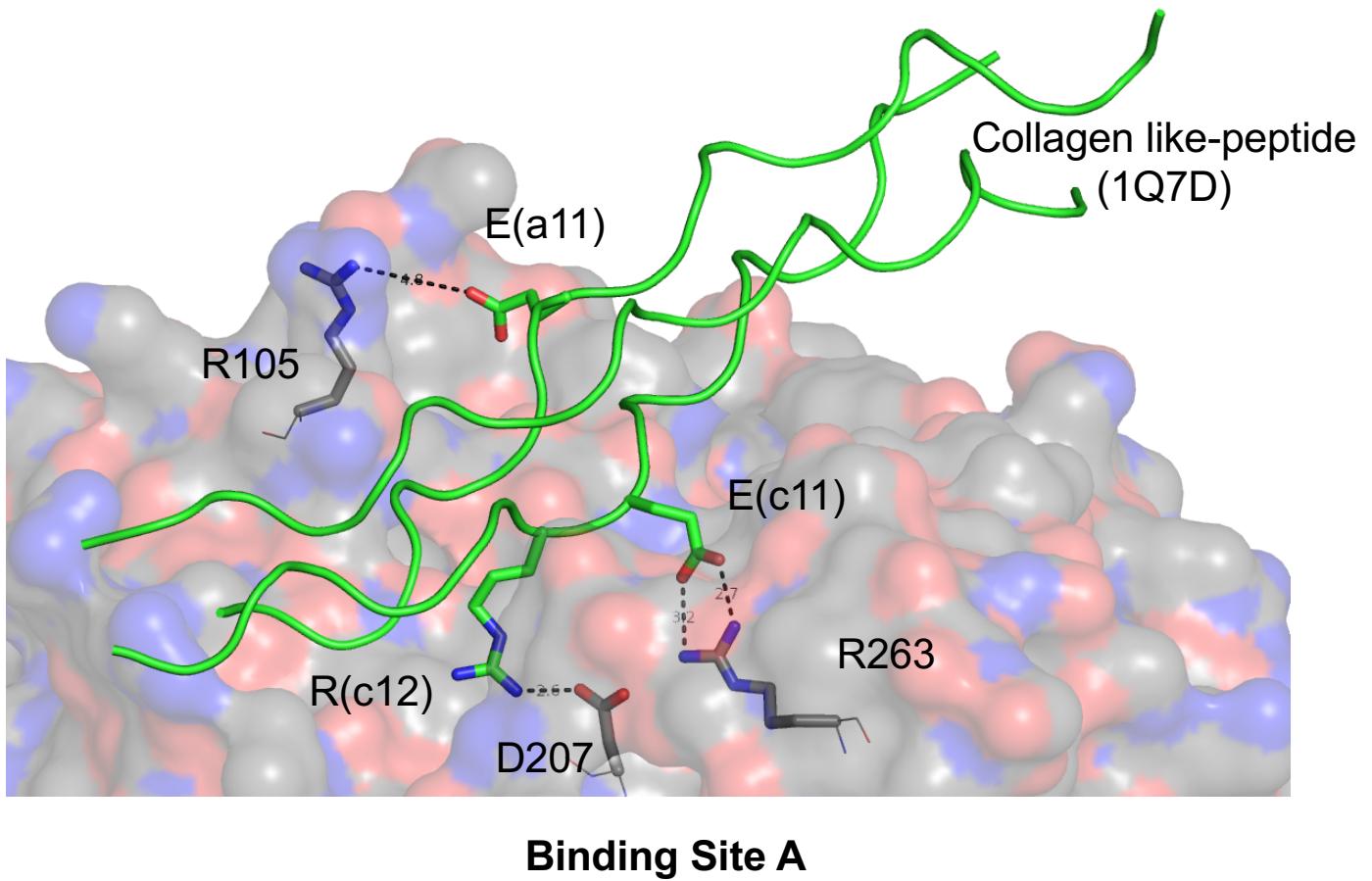
	Site A	Site B
ICAG	Unstable binding	Does not bind
ICAG_unmut	Stable binding	Stable binding
IBKV	Stable binding	Does not bind
IQ7D	Very stable binding	Very stable binding

Preferential Binding to Collagen With Integrin Binding Motif (GFOGER) at Site A

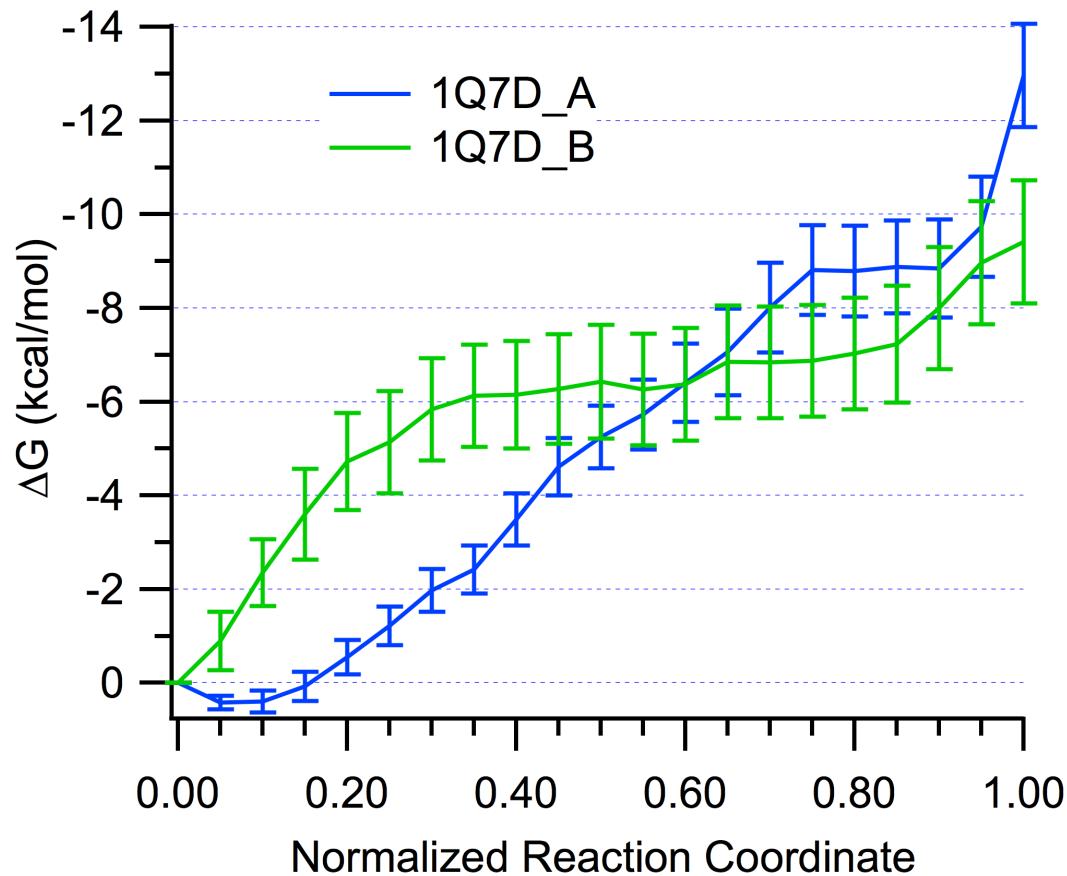


Potential of mean force (PMF) obtained from umbrella sampling MD simulations of the YKL-40-collagen system.

Interactions With GFOGER Motif

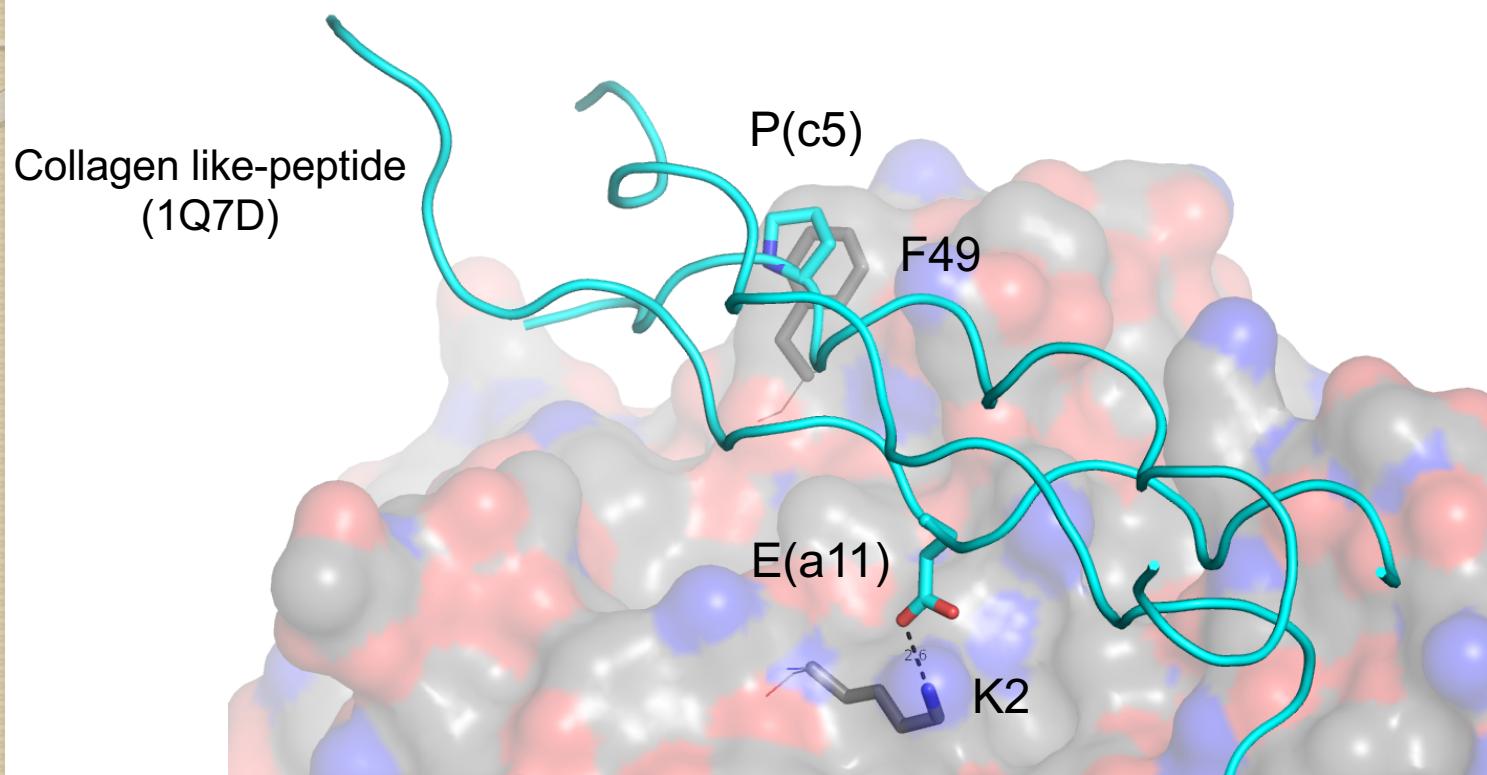


Affinity for Collagen at Site A vs Site B



Potential of mean force obtained from umbrella sampling MD simulations of the YKL-40-collagen system.

Collagen Binding at Site B



Binding Site B



Conclusions

- YKL-40 binds hyaluronan with the highest affinity, followed by chitin.
- Positively charged heparin binding domain responsible for non-specific surface binding.
- YKL-40 likely binds collagen at two possible sites through the formation of salt bridges and stacking interactions with Pro & Hyp.
- These findings not only identify potential physiological ligands of YKL-40 but also provide better viewpoint towards understanding the functions of YKL-40 in mammalian cells.



Acknowledgements

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Computational Resources



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- Suvamay Jana
- Dr. Inacrist Geronimo
- Yue Ye



Swedish University of
Agricultural Sciences

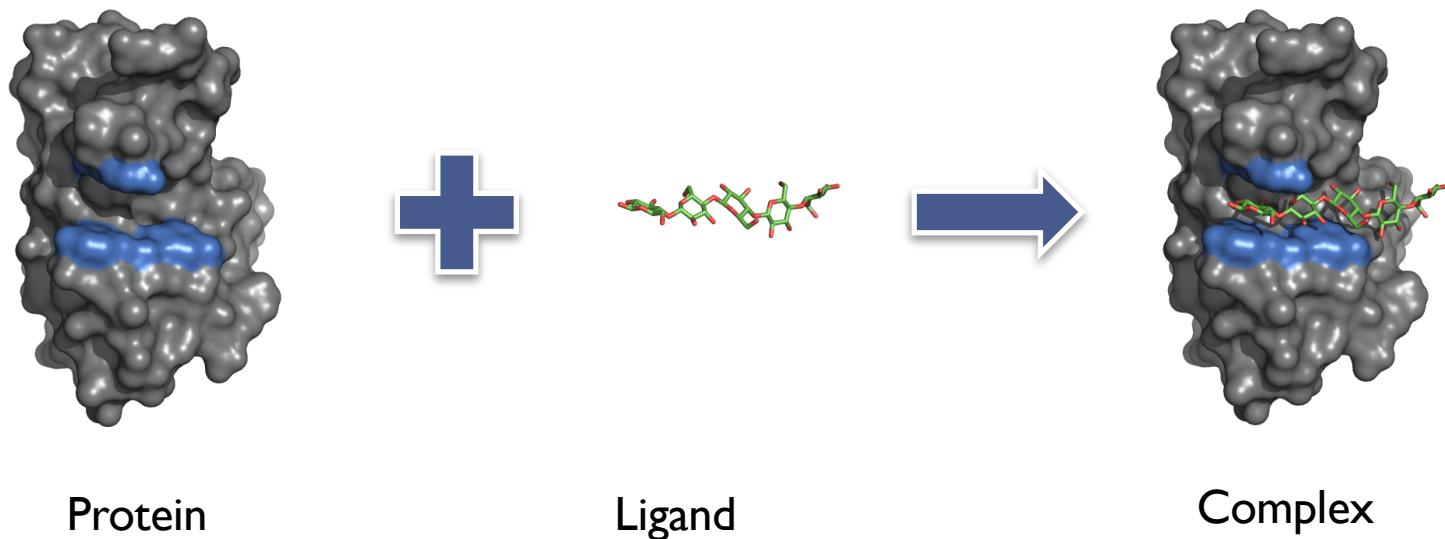


Questions?



Extra Slides

Thermodynamics of Carbohydrate Recognition



$$\Delta G = -RT \ln(K_a) = \Delta H - T\Delta S$$

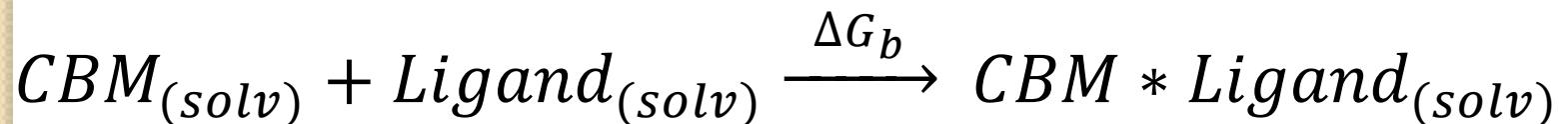
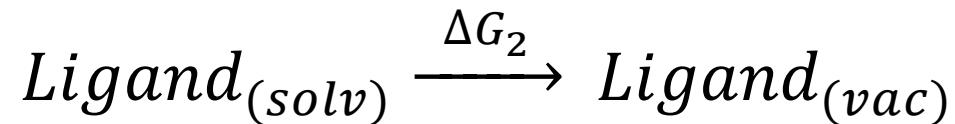
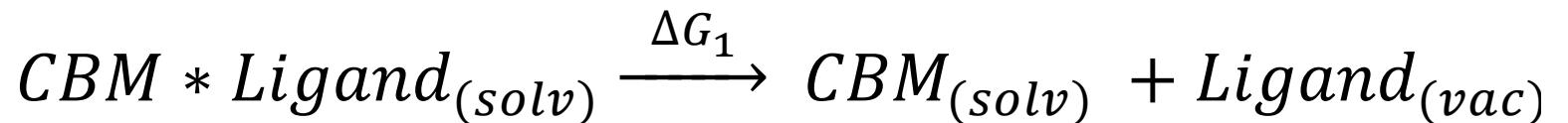
ΔH (enthalpic contribution)	ΔS (entropic contribution)
<ul style="list-style-type: none">• VDW• Coulombic• Hydrogen bonding	<ul style="list-style-type: none">• Loss of translational and conformational freedom• Solvation effects

- VDW
- Coulombic
- Hydrogen bonding

- Loss of translational and conformational freedom
- Solvation effects

Ligand Binding Free Energy Calculation

Free Energy Perturbation
with Replica Exchange Molecular Dynamics
(FEP/λ-REMD)



$$\Delta G_b = \Delta G_2 - \Delta G_1$$

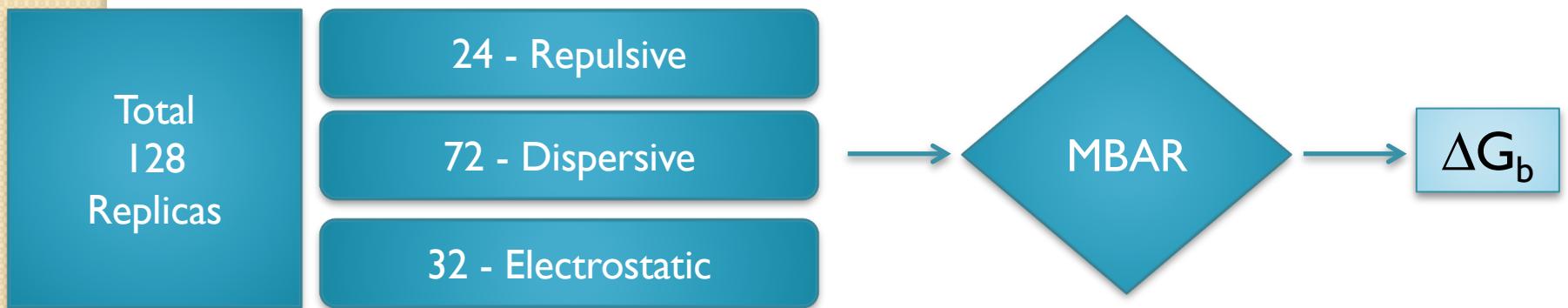
FEP/λ-REMD

- Free Energy Perturbation

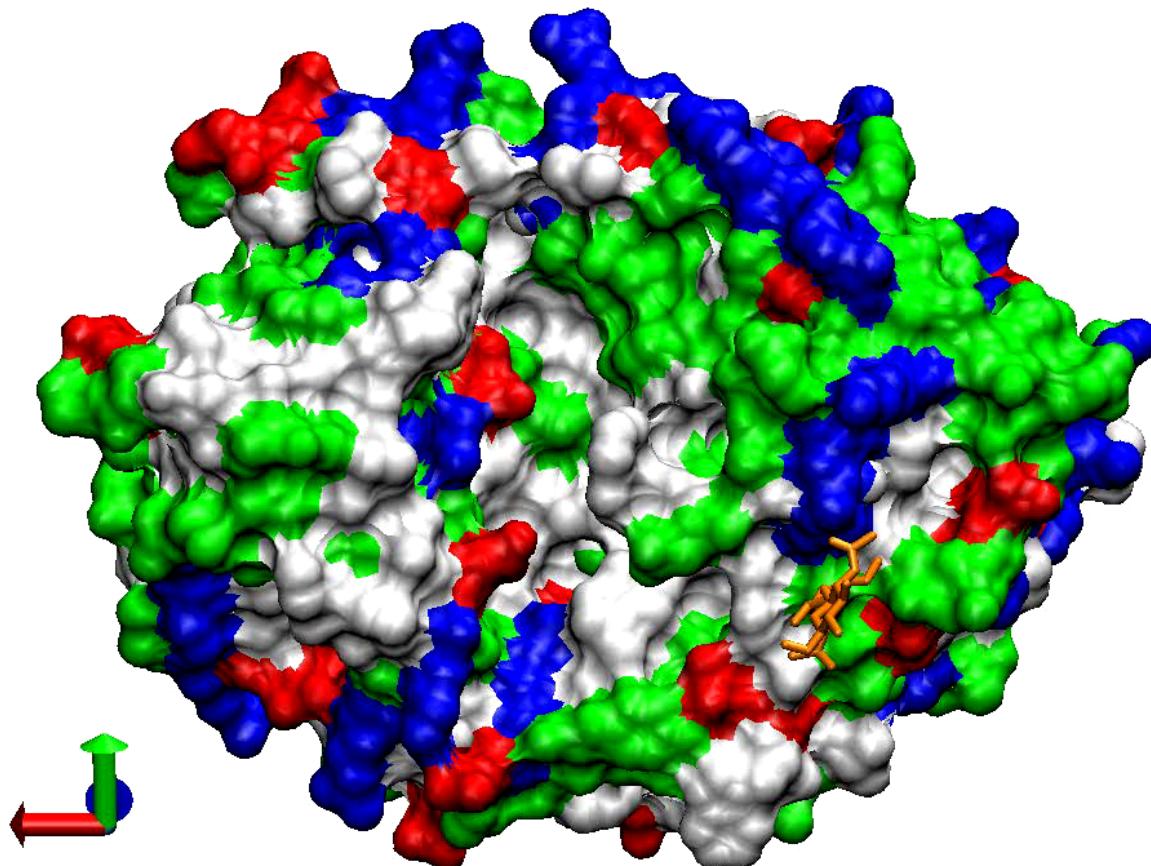
$$\Delta G(A \rightarrow B) = G_B - G_A = -k_B T \ln \langle \exp \left(-\frac{E_B - E_A}{k_B T} \right) \rangle_A$$

- Replica Exchange Molecular Dynamics

$$U = U_o + \lambda_{rep} U_{rep} + \lambda_{dis} U_{dis} + \lambda_{elec} U_{elec} + \lambda_{rstr} U_{rstr}$$



YKL-40 – Surface – Residue Type



Native Contacts for Umbrella Sampling

$$p(i) = \text{weight of contact} = \frac{\text{No. of frames it's present in}}{\text{Total no. of frames}}$$

$$\text{State of Contact} = x(i) = \frac{1}{(1 + \exp(20 \cdot (d(i) - 12.0)))}$$

$$\text{Reaction Coordinate } (\rho) = \frac{\sum p(i) \cdot (1 - x(i))}{\sum p(i)}$$