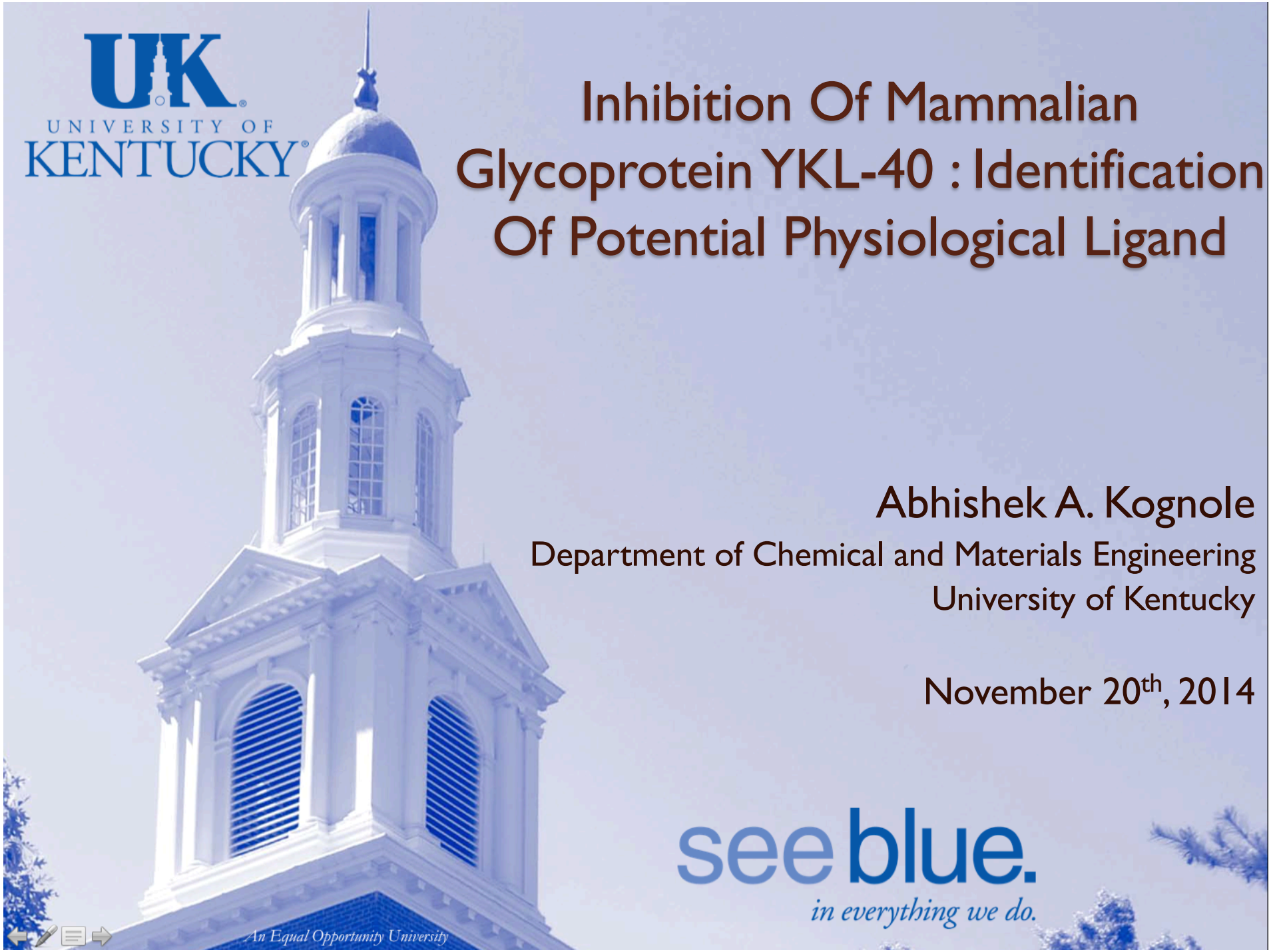

Inhibition Of Mammalian Glycoprotein YKL-40 : Identification Of Potential Physiological Ligand

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Department of Chemical and Materials Engineering
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seeblue.
in everything we do.

Introduction to YKL-40

- Categorized as non-catalytic mammalian glycoprotein

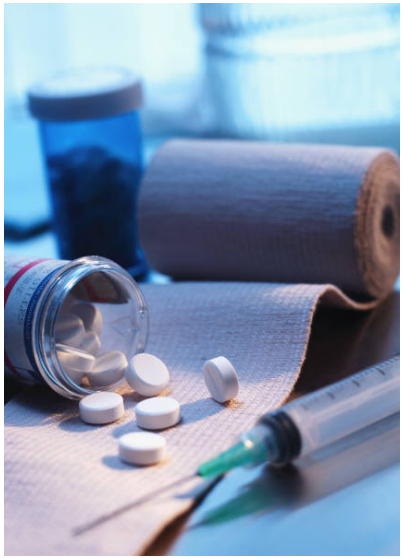
Glycoprotein	Disaccharide of GlcNAc covalently bound at N59
Mammalian	*In vivo* secretion by synovial cells, chondrocytes, endothelial and epithelial cells, and tumor cells in mammals.
Non-catalytic	• Classified as Glycoside Hydrolase (GH) Family 18 chitinases based on protein sequence • Substitutions in motif essential for catalysis DXXDXDXE → DXXDXAXL • Lectin – a non-catalytic sugar binding protein

- Also known as CHI3L1 (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 it's role in tissue remodeling leaves it as an potential therapeutic target in several disorders.

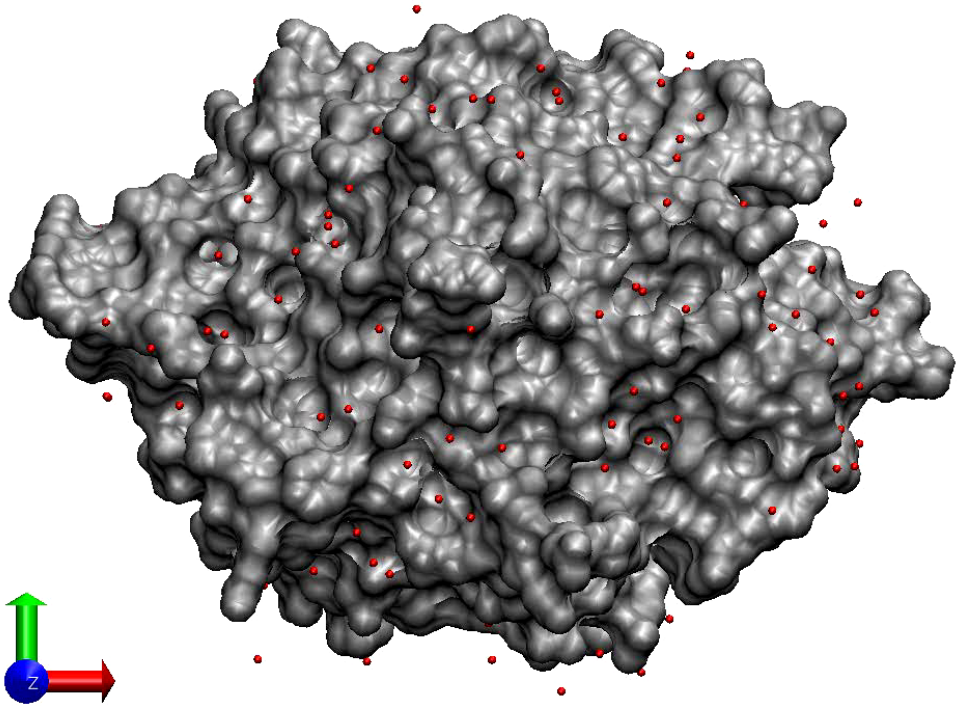


Binding Sites of YKL-40

1

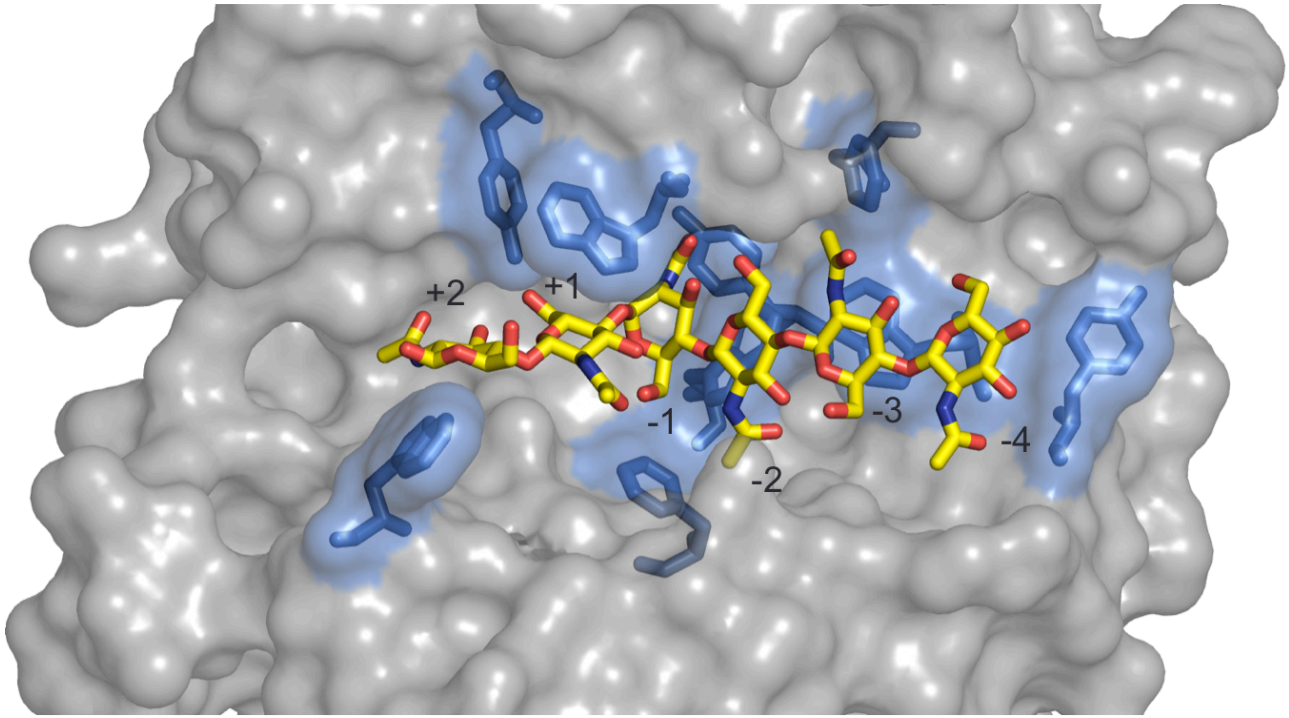
2?

More ?



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

Traditional Binding Cleft for Carbohydrates

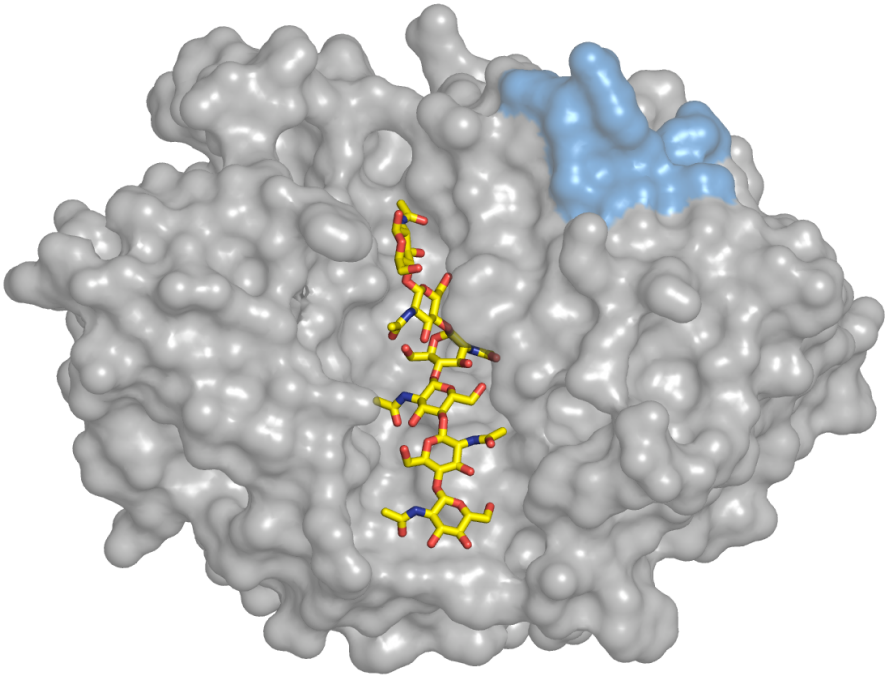


- Chitin is been reported as natural substrate from X-ray crystallographic study
- Chitin – polysaccharide of N-acetyl glucosamine (GlcNAc)
- Binding cleft with 9 sugar-binding subsites from +3 to -6
- Potential binding site for similar carbohydrates?

Houston et al., J. Bio. Chem. (2003)

2

Putative Surface Binding Site for Heparin

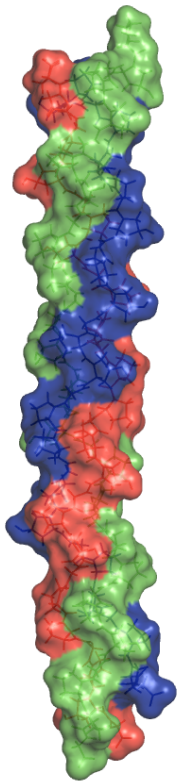


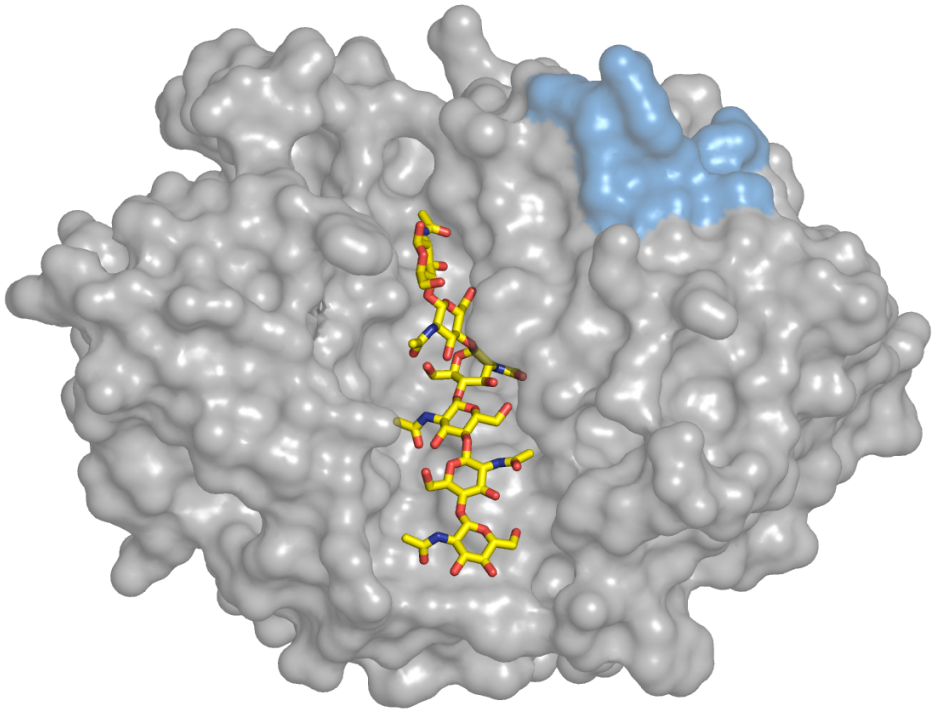
- Protein purification of YKL-40 uses its binding affinity for heparin
- Heparin – Highly sulfated carbohydrate and is part of Extra Cellular Matrix (ECM) along with YKL-40.
- No structural evidence of Heparin bound at the binding site.
- Complimentary features of heparin and protein surface?

Fusetti et al., J. Bio. Chem. (2003)

More ?

Protein-protein Interaction of YKL-40

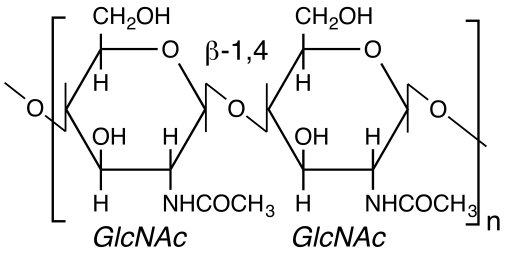




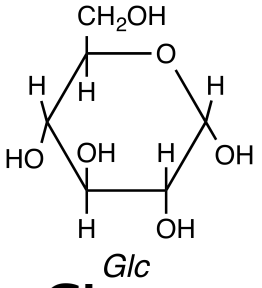
- Specific binding affinity for three types of collagen
- Collagen – triple helical protein also comprises most of ECM
- Ambiguous effects of YKL-40 binding on fibril formation of collagen
- No binding site characterization with lack of structure of bound complex

Bigg et al., J. Bio. Chem. (2006)

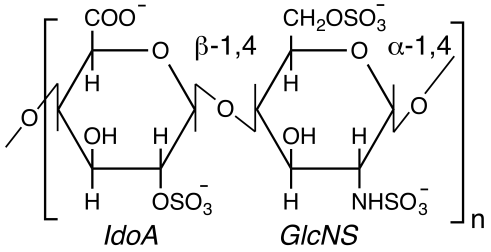
Potential Carbohydrate Ligands



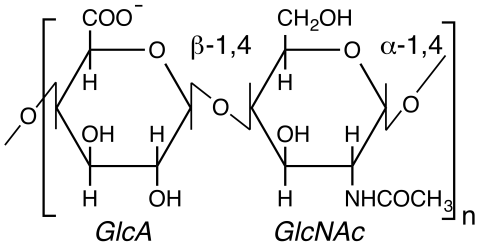
Chitin



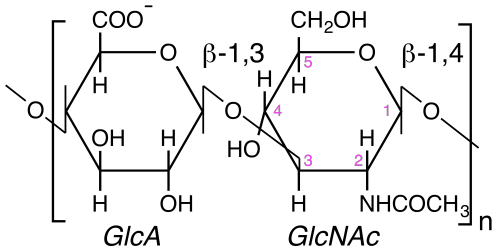
Glucose



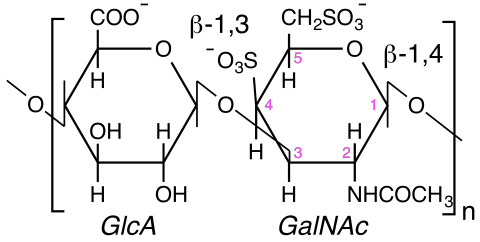
Heparin



Heparan Sulfate



Hyaluronan

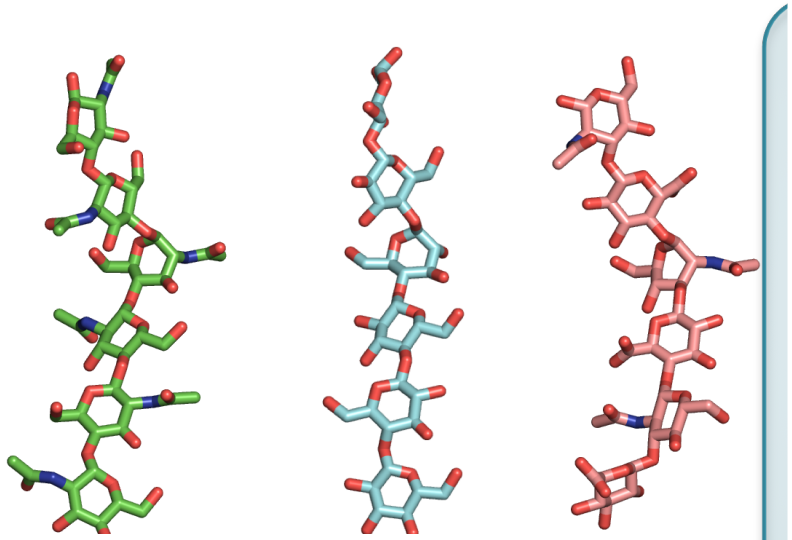


Chondroitin Sulfate

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 with 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

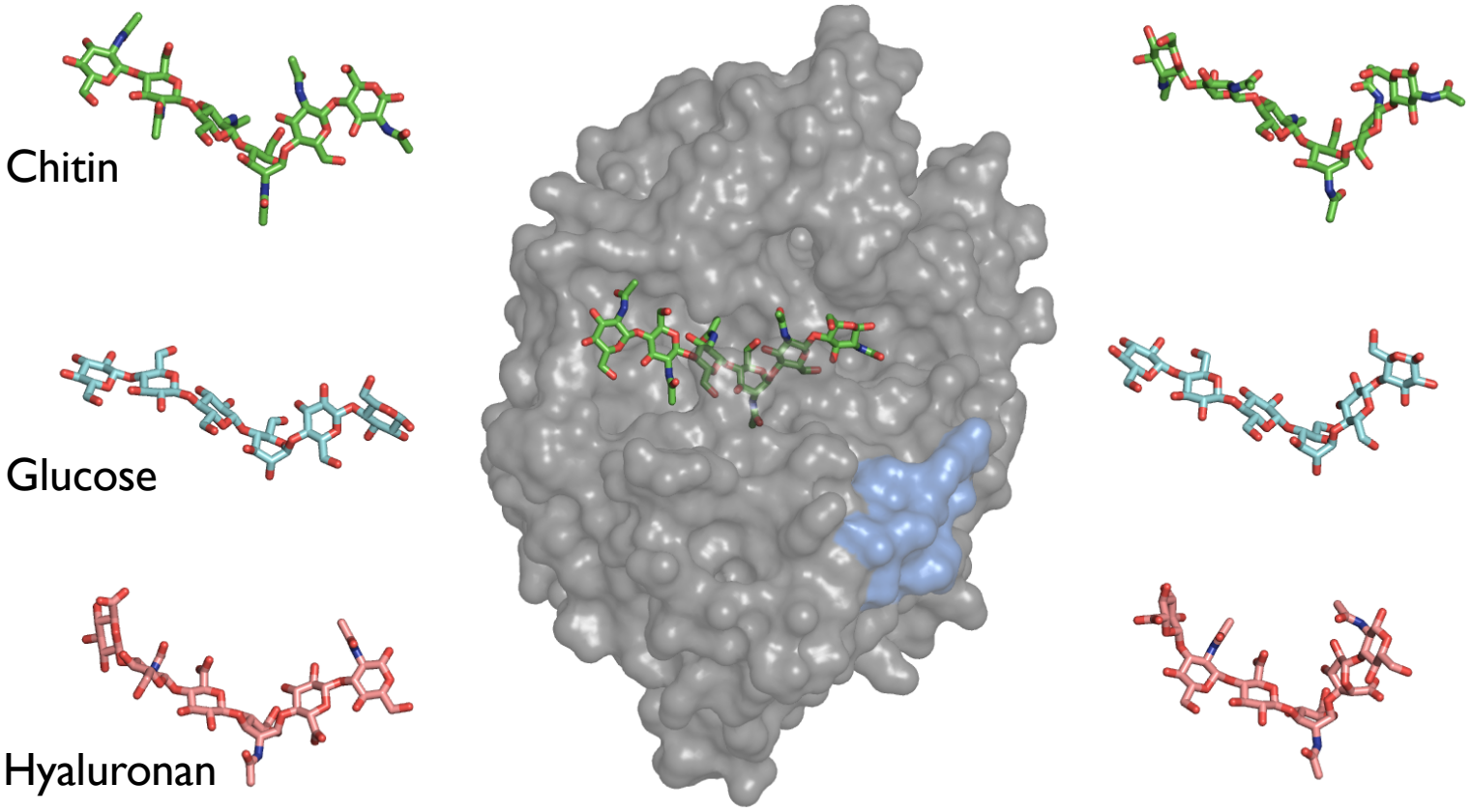


Chitin

Chitin Glucose Hyaluronan

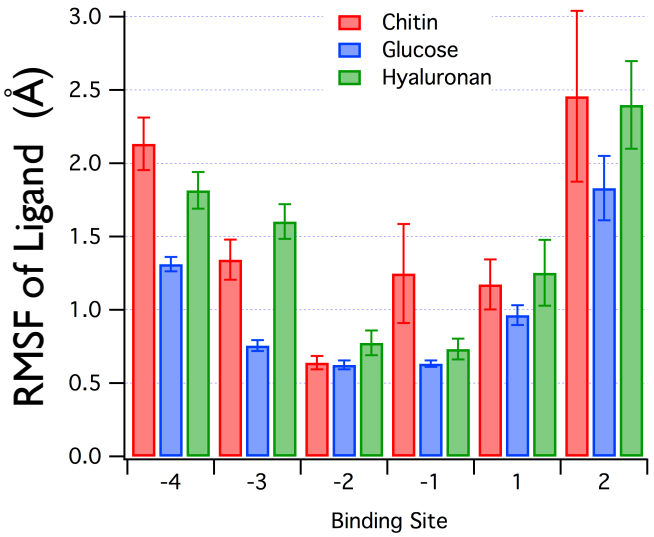
Exit the binding cleft

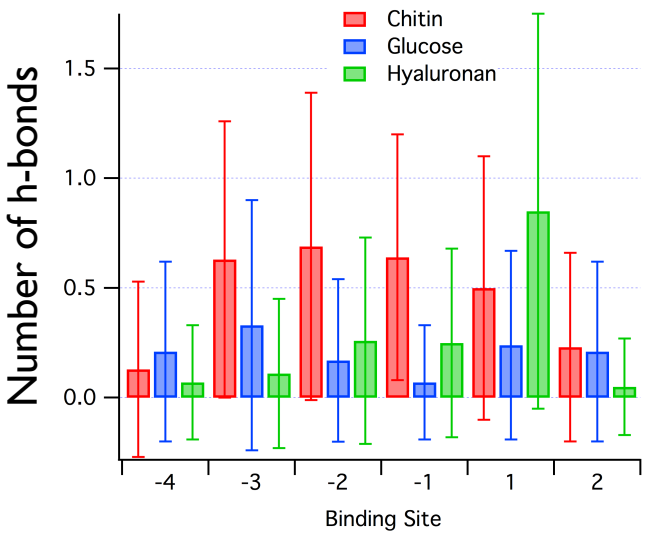
Who Is Welcome In The Cleft?

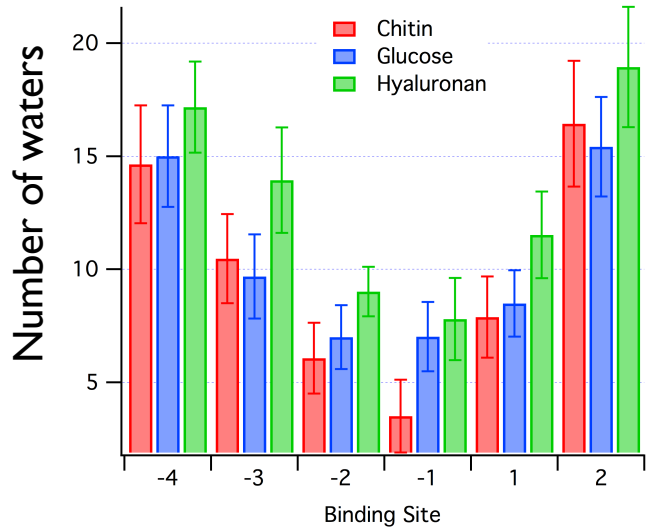


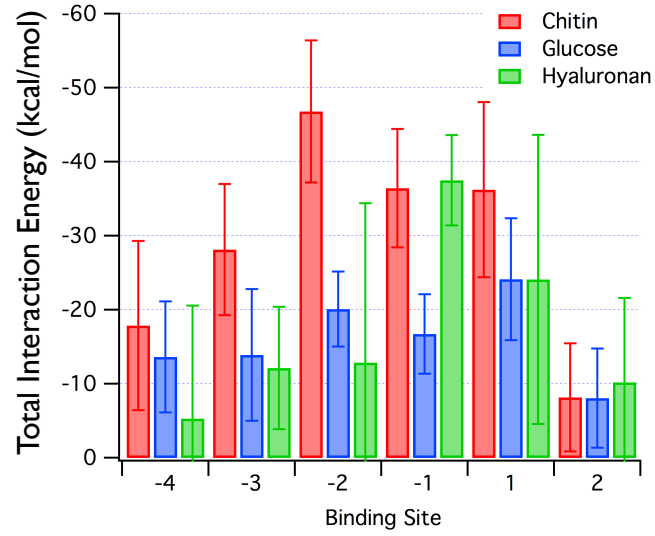
Hyaluronan : Potential Physiological Ligand ?

Analysis of Polysaccharide Binding Dynamics



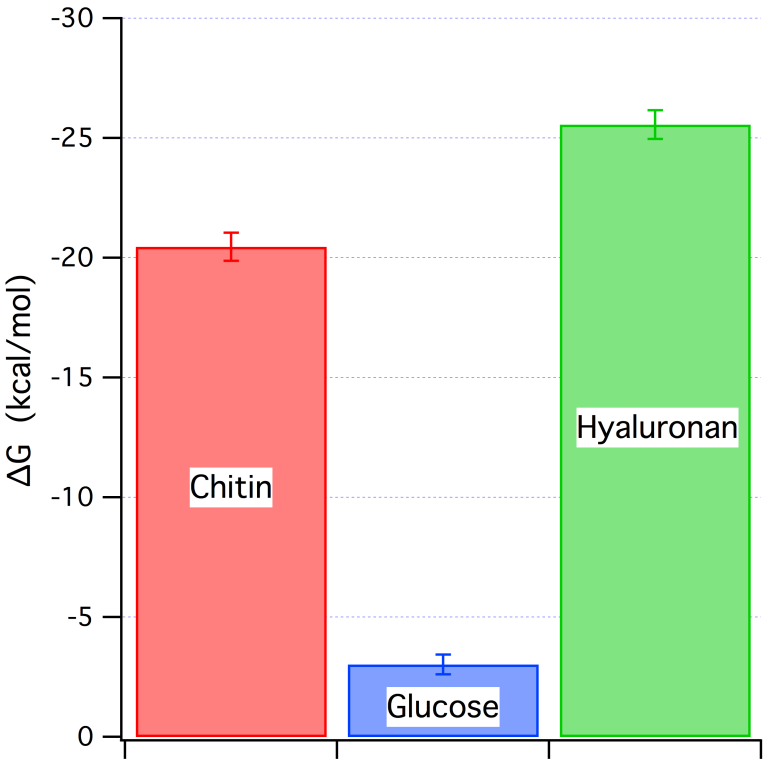






Hyaluronan : Potential Physiological Ligand ?

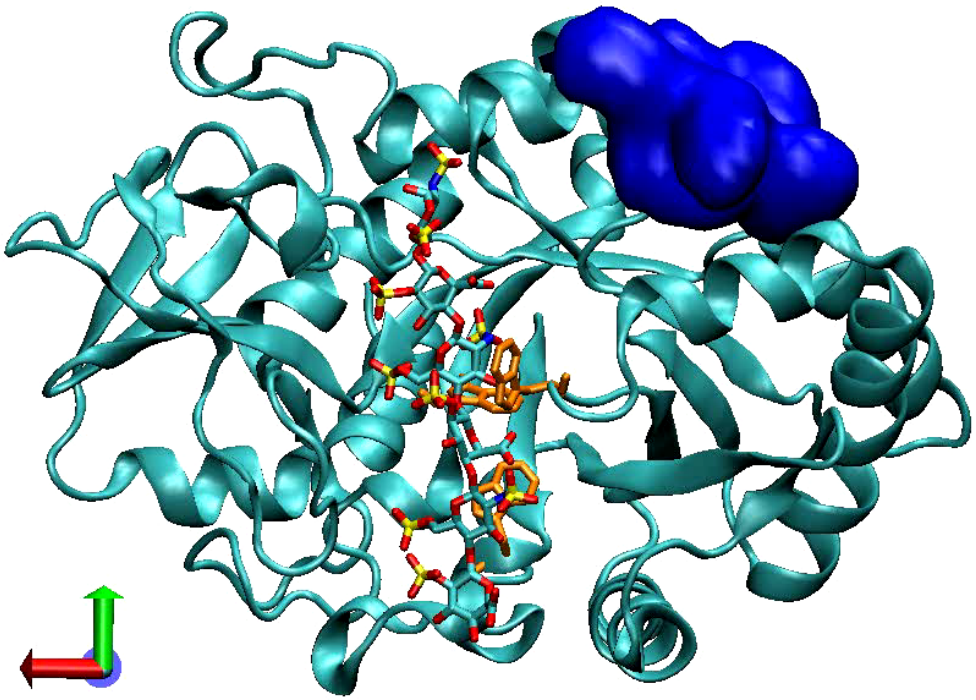
Relative affinity in terms of absolute binding free energy



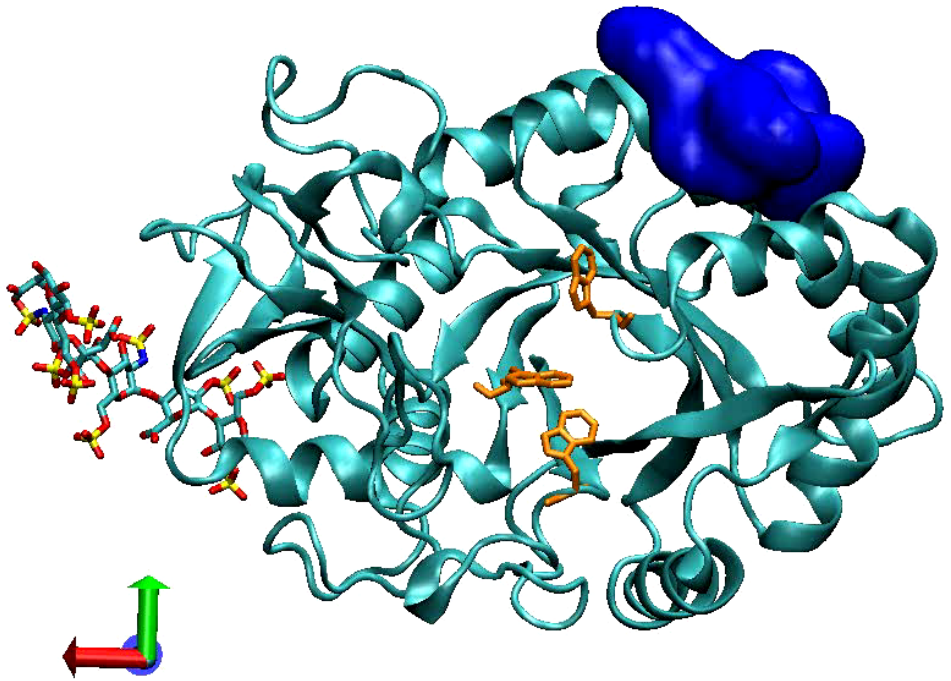
Absolute binding free energy of chitin, glucose, and hyaluronan to YKL-40 calculated by FEP/λ-REMD method.

- Glucose (cello-oligosaccharide) out of race due to low potential for enthalpic contribution.
- Chitin being natural substrate demonstrates ΔG resembling to that of family 18 chitinases.
- Hyaluronan shows most of the enthalpic contributions from chitin also according to binding dynamics
- Negative charge on hyaluronan appears to be additionally contributing through electrostatic contributions and solvation effects.

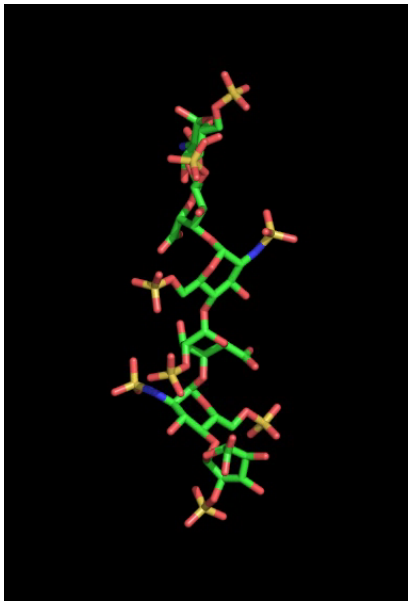
What Happens To Heparin?



Starting From Different Coordinates!



What is this site comprised of ?



Solution structure of Heparin

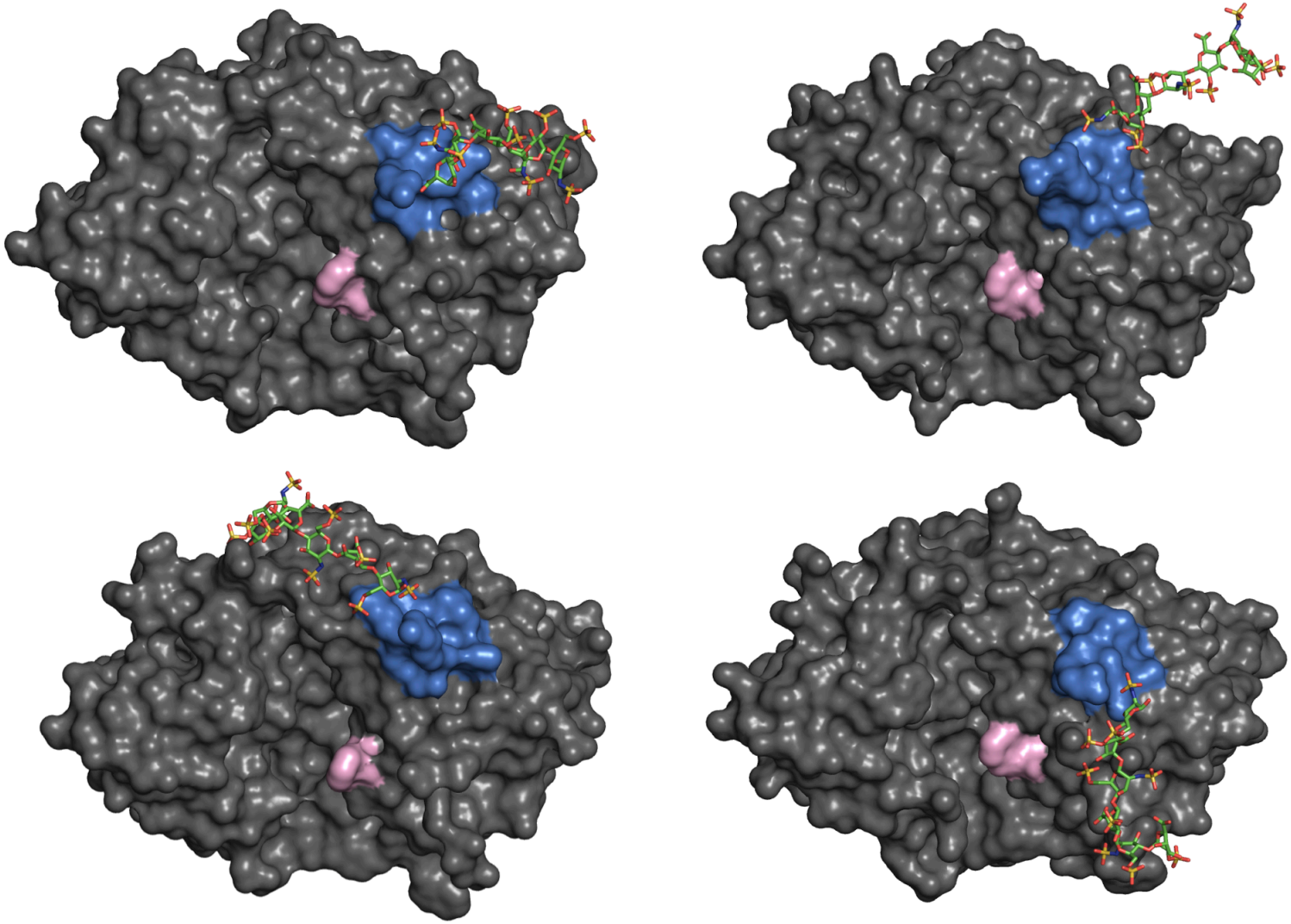
- Comparison with Heparin-binding consensus sequences

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.

Cardin and Weintraub, Arteriosclerosis (1989)

Need Of Statistically Significant Conformation



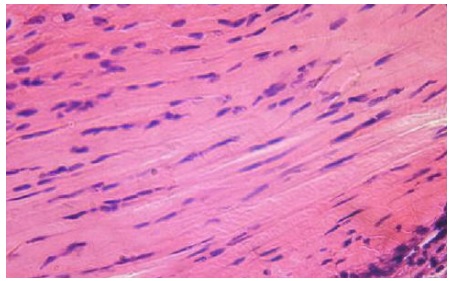
Heparin (green stick representation)

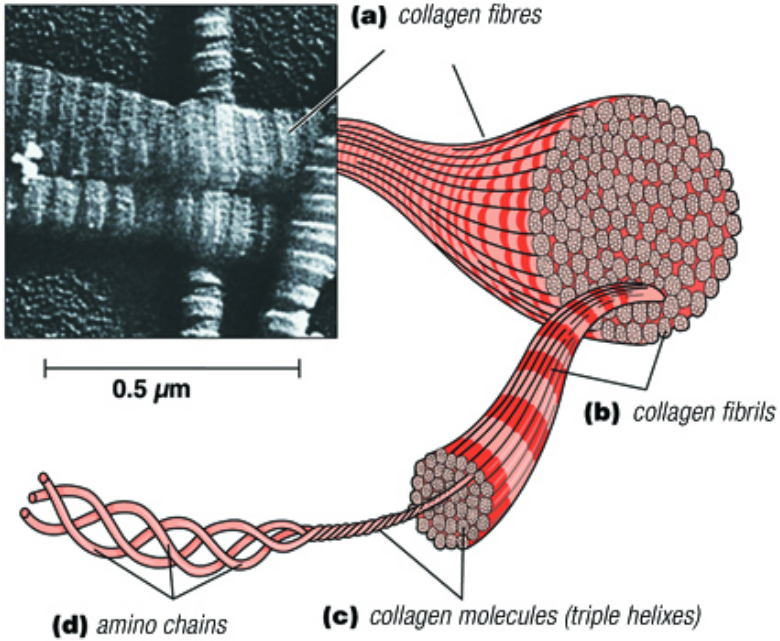
Putative heparin-binding site of YKL-40 (blue surface representation)

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.
- Different isoforms of YKL-40 extracted from cartilage and chondrocytes display ambiguous effects on collagen fibril formation.

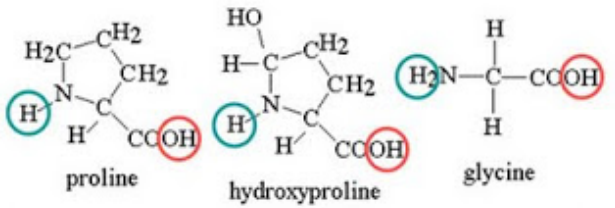


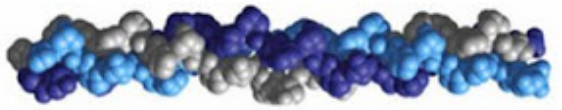


Bigg et al., J. Bio. Chem. (2006)

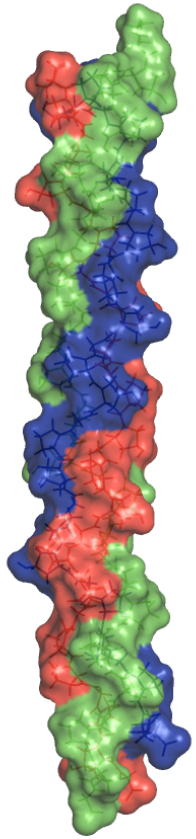
Image: http://www.sciteconline.com

Collagen :





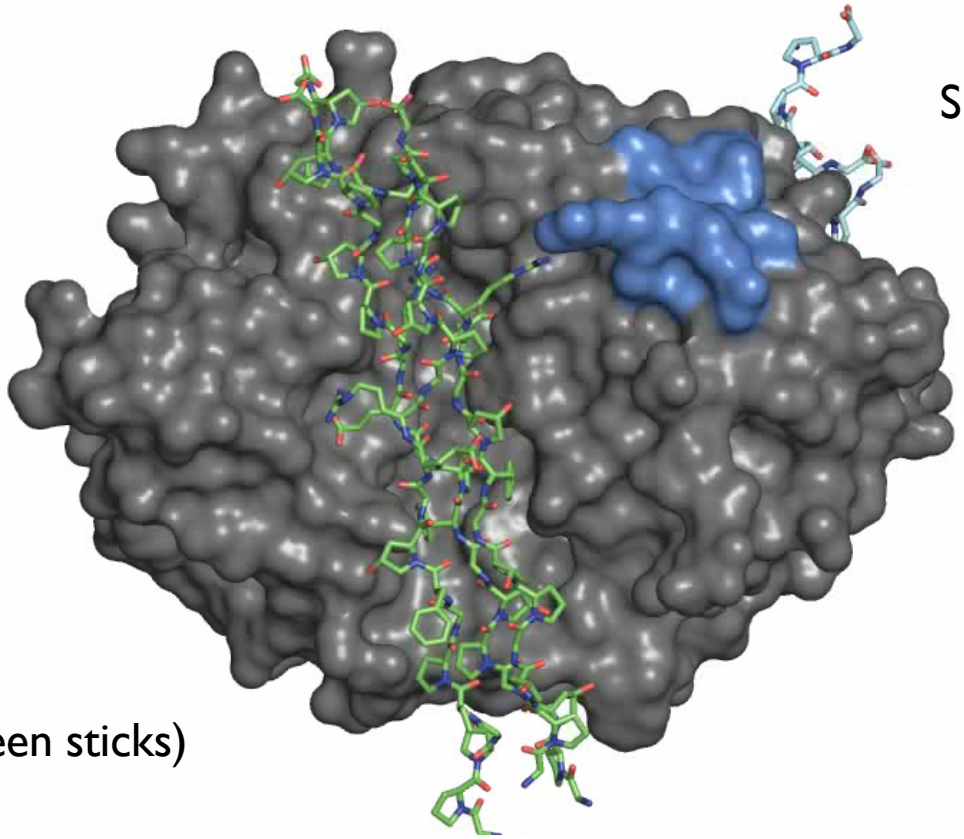
- Collagen is macromolecular protein with triple helical structure
- basic Gly – Pro – Hyp repeating amino acid sequence
- 27 different types of collagen
- 10/3 and 7/2 symmetries of helix
- Selection of 4 model collagen peptides to represent collagen in entirety



Collagen: Representative Peptide Models

- 1CAG
  - Basic model consisting only Gly-Pro-Hyp repeatedly, with one substitution of Gly → Ala and relaxed 7/2 symmetry
- 1CAG_unmut
  - Same as 1CAG model without substitution with perfect 7/2 symmetry.
- 1BKV
  - Collagen-like model peptide consisting sequence, GITGARGLA, in middle from human type III collagen with 10/3 symmetry
- 1Q7D
  - Model peptide consisting GFOGER motif known to bind the integrin α2β1-I receptor protein with mixed symmetry

Where Is The Binding Site For Collagen?

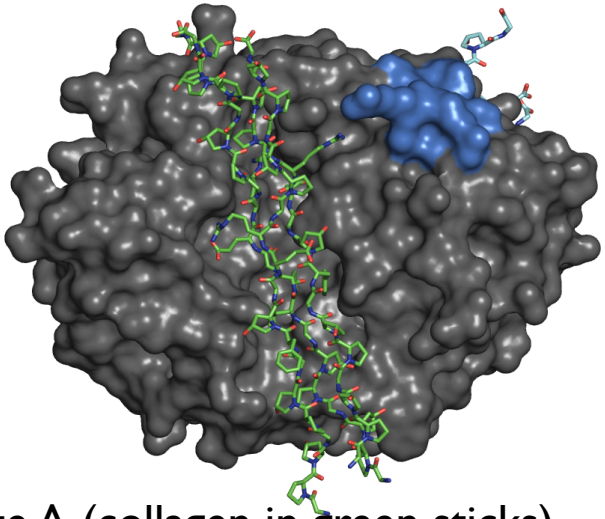


Site B (cyan sticks)

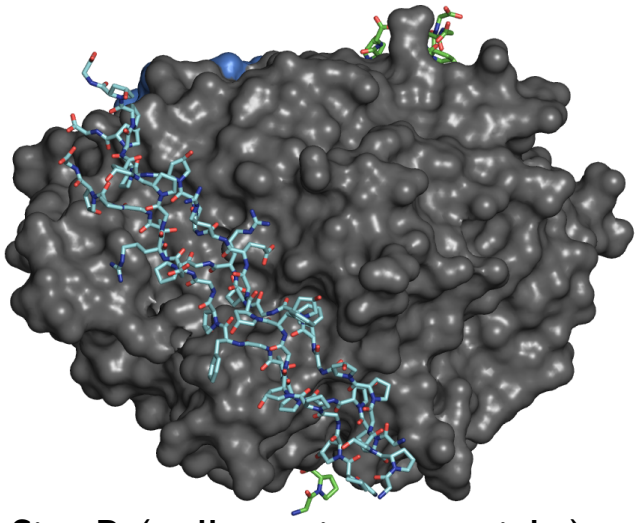
Site A (green sticks)

Molecular shape complementarity docking calculations predict collagen-like peptides will bind to YKL-40 in two possible orientations.

Binding Dynamics at Site A and Site B



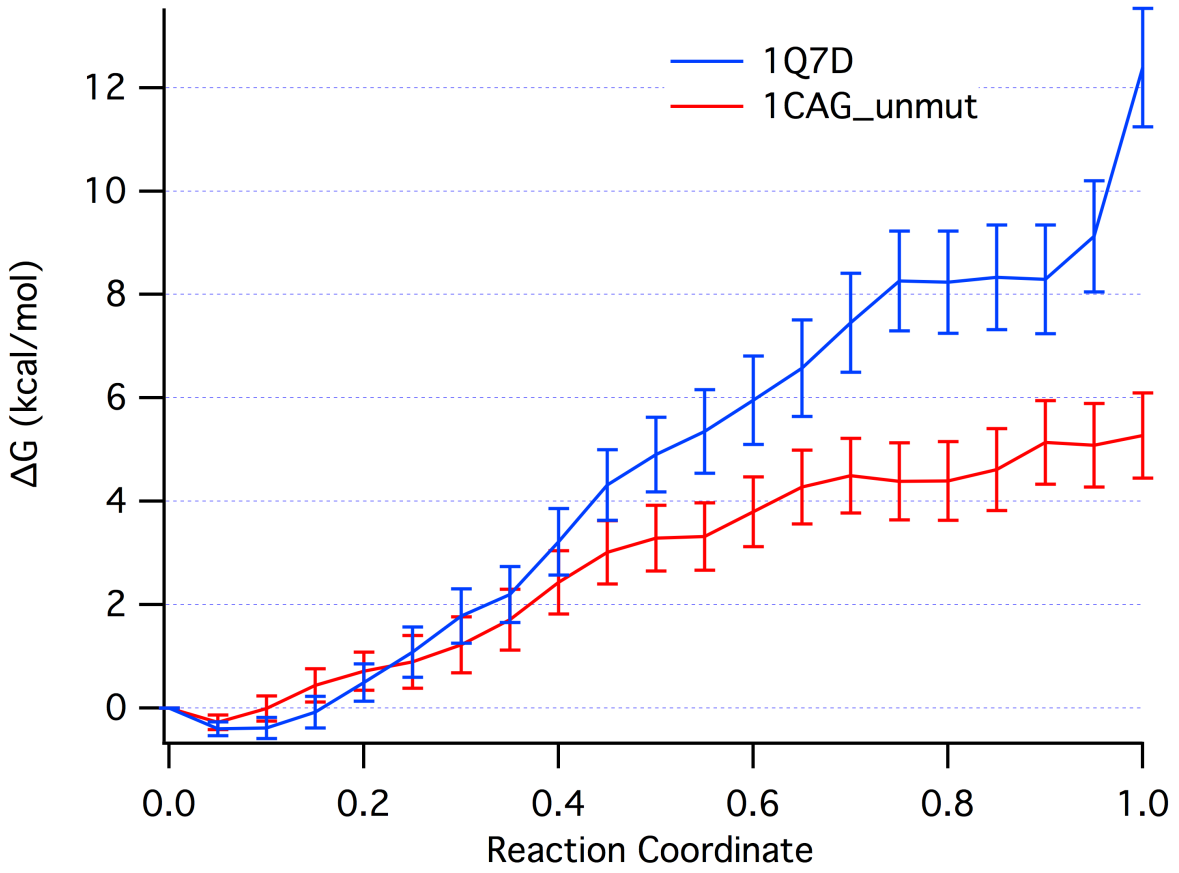
Site A (collagen in green sticks)



Site B (collagen in cyan sticks)

	Site A	Site B
1CAG	Unstable binding	Does not Bind
1CAG_unmut	Very Stable binding	Stable binding
1BKV	Stable binding	Unstable binding
1Q7D	Very stable binding	Stable binding

Preferential Binding to Integrin Binding motif



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

Conclusions

- Chitin and hyaluronan appear to be preferential physiological ligands of YKL-40.
- Confirmation of the non-specific interaction of heparin with the putative heparin-binding domain as suggested by previous studies.
- Probable binding sites for collagen and comparison for dynamics and affinity over different models adds to knowledge about diverse functions of YKL-40.
- These findings not only identify physiological ligands of YKL-40, they enable future efforts to rationally guide design of YKL-40 inhibitors.

Acknowledgements

Funding

KENTUCKY SCIENCE AND ENGINEERING FOUNDATION

Computational Resources

CCS
Center for Computational Sciences

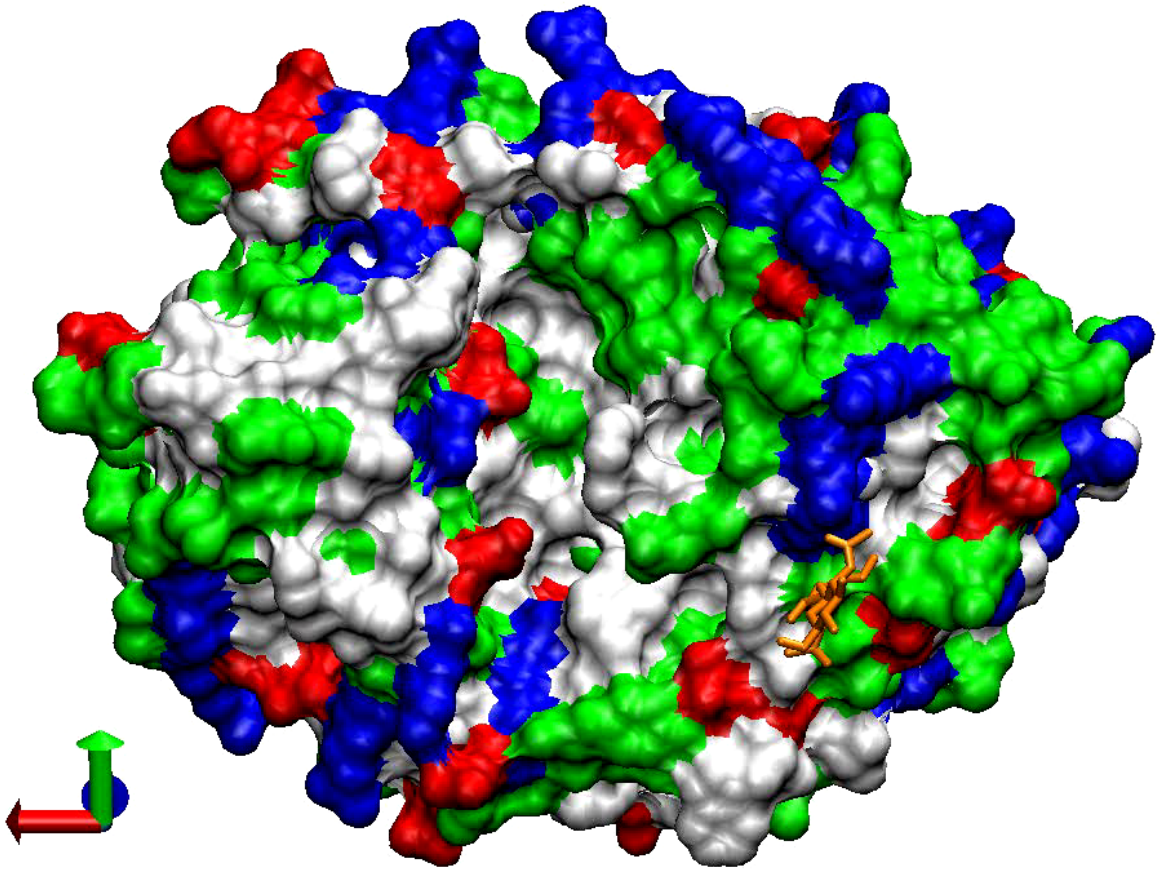
XSEDE
Extreme Science and Engineering Discovery Environment

Principal Investigator : Dr. Christina M. Payne

Suvamay Jana

Extra Slides

YKL-40 – Surface – Residue Type



Native Contacts for Umbrella Sampling

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

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

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