Inhibition of Mammalian Glycoprotein YKL-40: Identification of Physiological Ligand

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Ashland Inc. Distinguished Lectures & Symposium on Drug Discovery and Development Chapter
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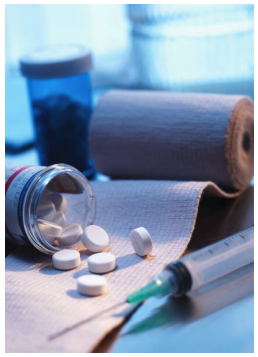
Inhibition of YKL-40 : Identification of Potential Physiological Ligand

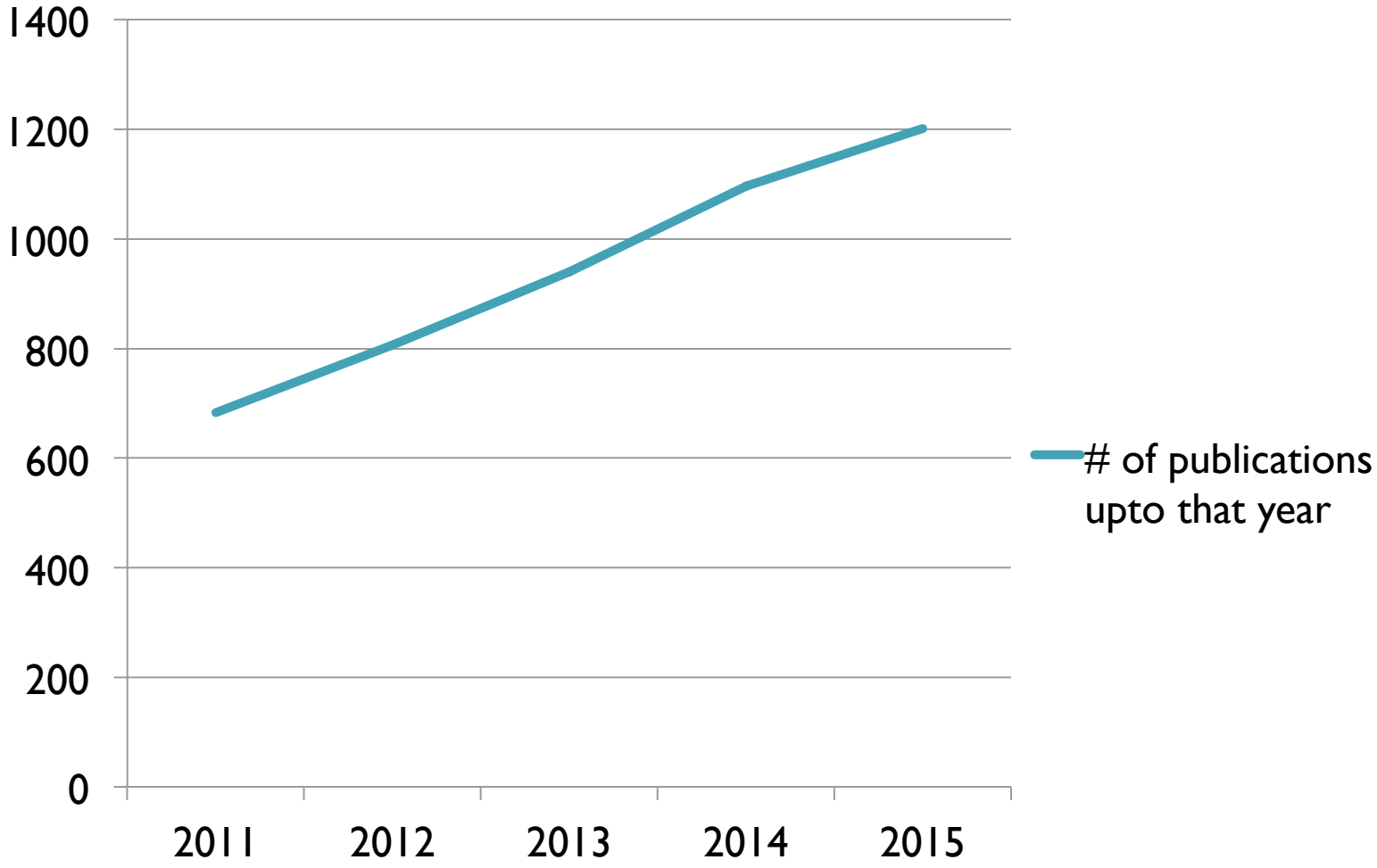
Mammalian	Glycoprotein	Non-catalytic

- 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.



WebofKnowledge: TOPIC=YKL-40

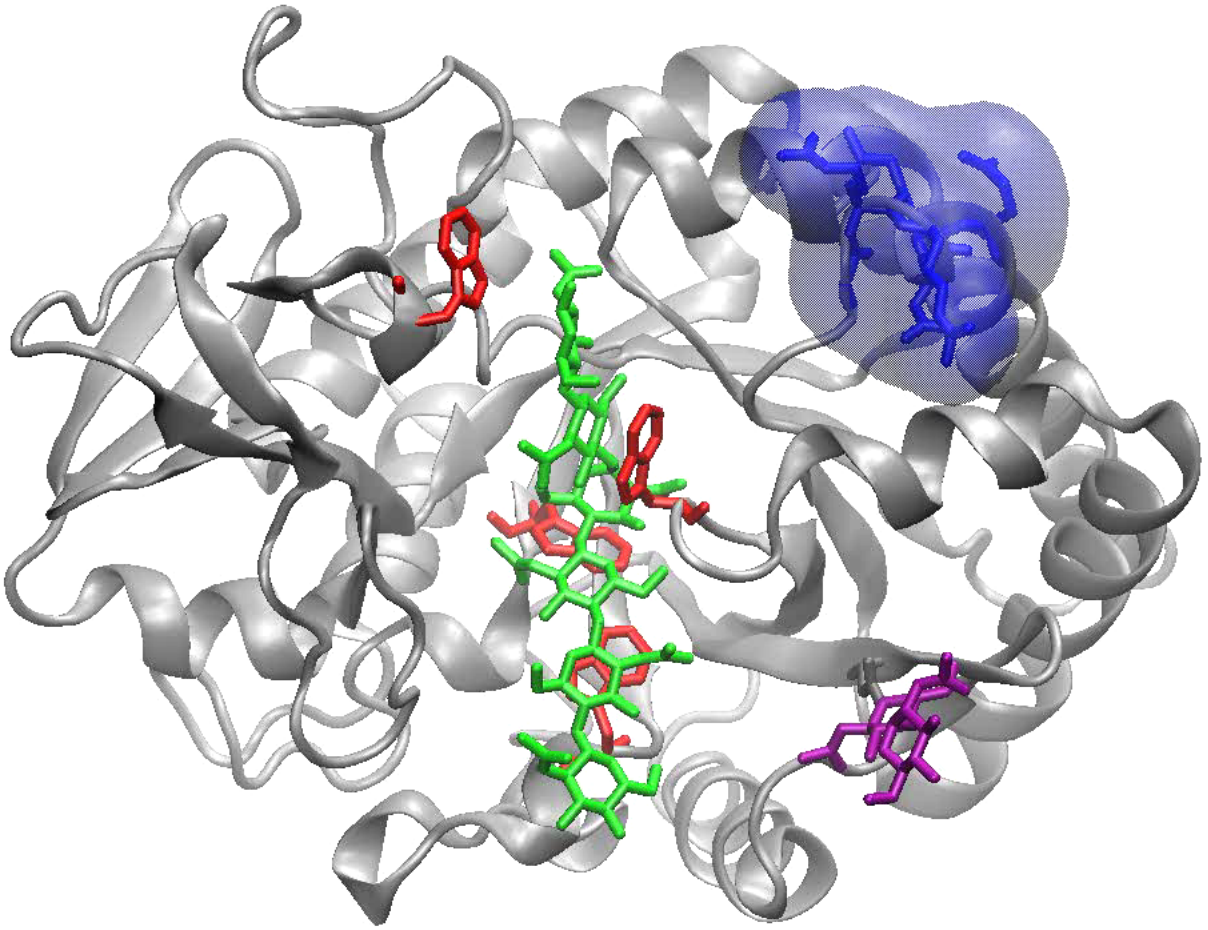


Binding Sites of YKL-40

1

2

More ?



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

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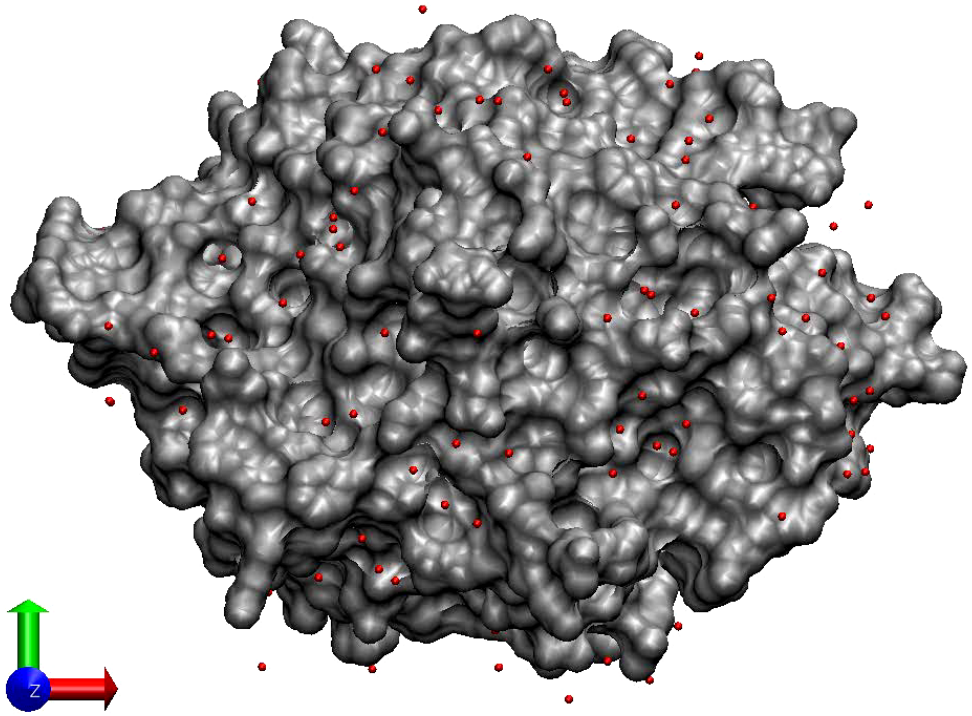
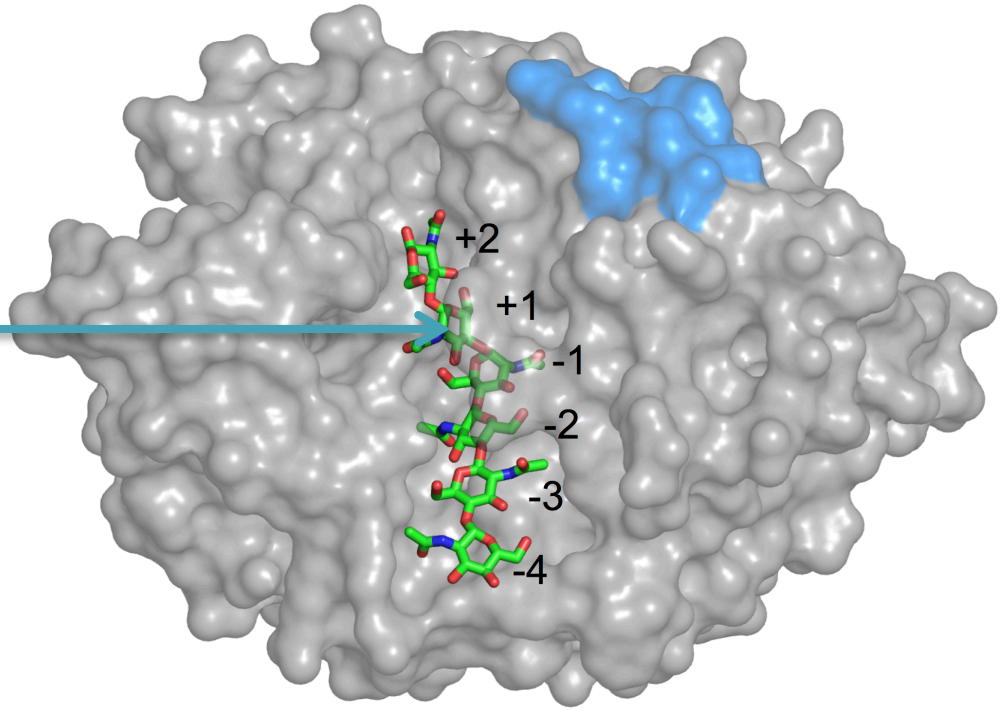


Chitin-bound YKL-40 structure by Houston et al., J. Bio. Chem., (2003)
PDB ID : 1HJW

Carbohydrate Binding Site

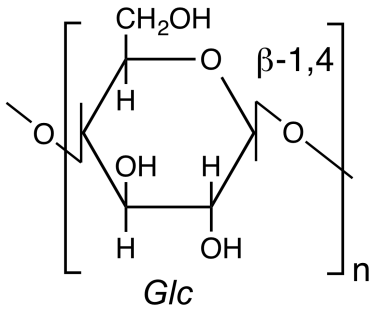


- YKL-40 has been shown to bind chito-oligosaccharides with X-ray crystallographic study
- **Chitin** – polysaccharide of N-acetyl glucosamine (GlcNAc)
- Binding cleft with 9 sugar-binding subsites from +3 to -6
- Potential binding site for similar carbohydrates?

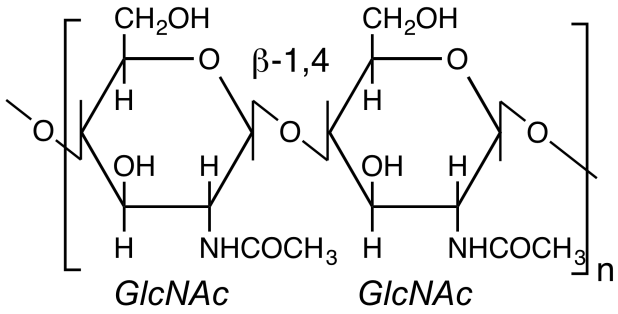
Houston et al., J. Bio. Chem., (2003)

Potential Carbohydrate Ligands

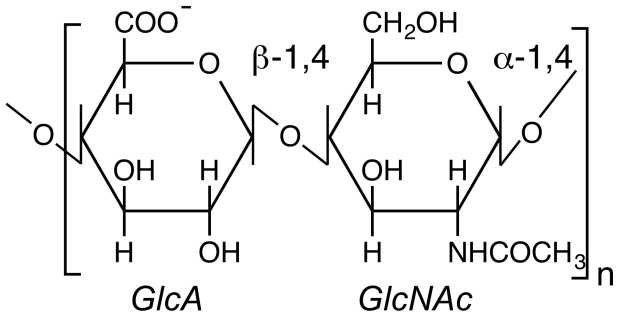
Cello-oligomer



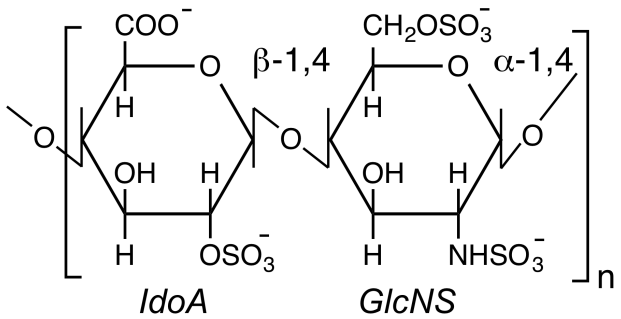
Chito-oligomer



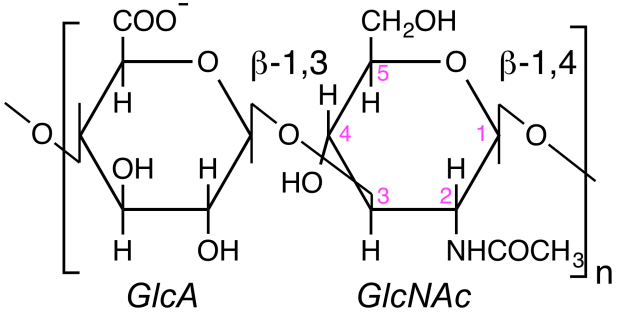
Heparan Sulfate



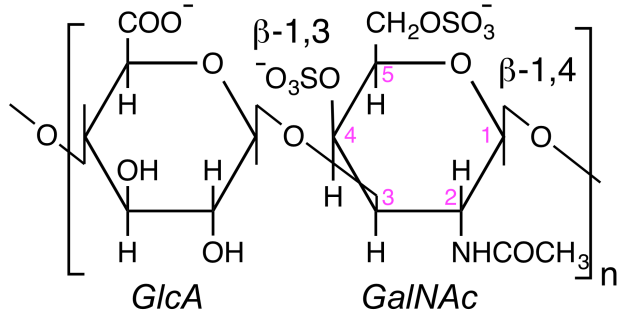
Heparin



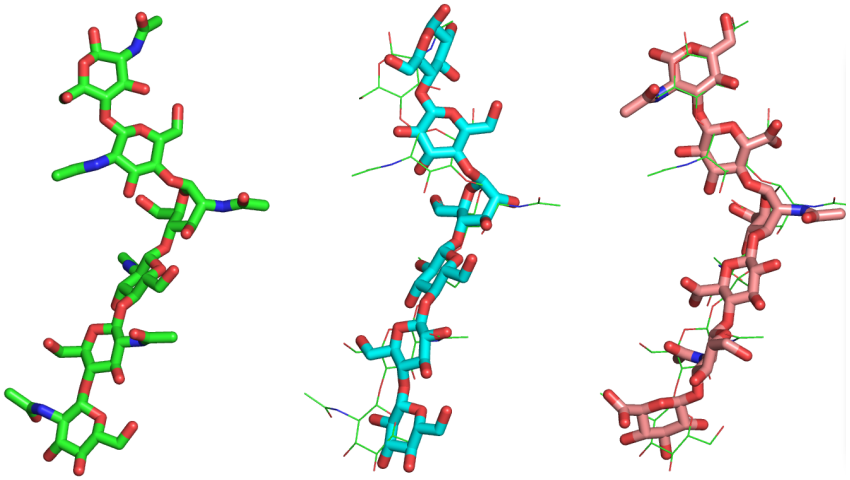
Hyaluronan



Chondroitin Sulfate



Over the 250 ns simulation

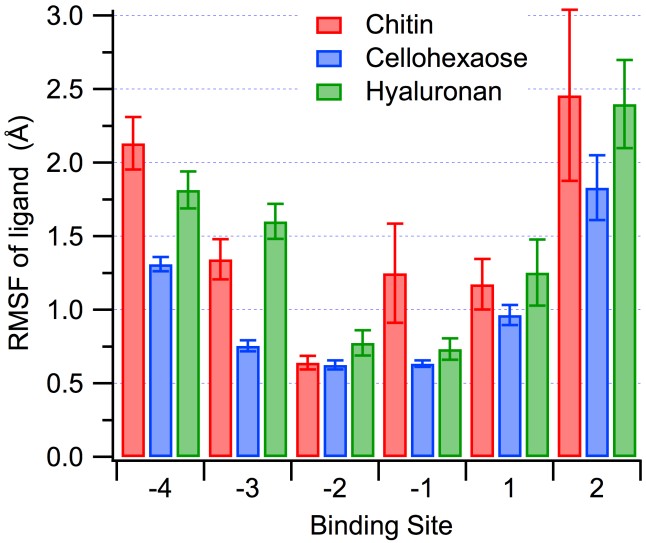


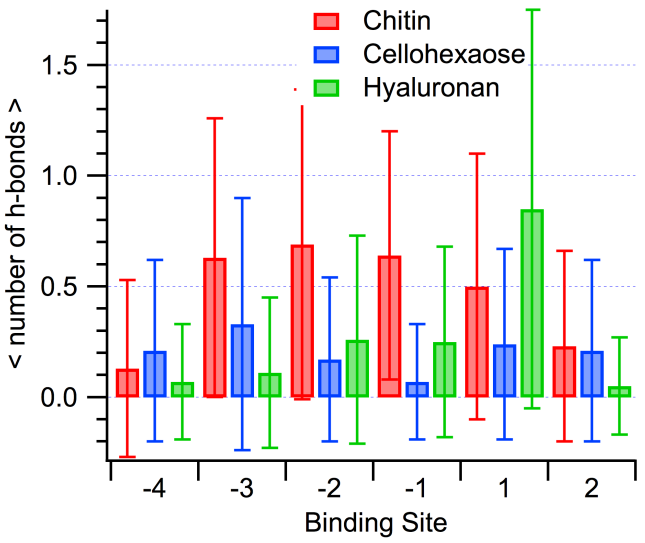
Chitin Cellohexaose Hyaluronan

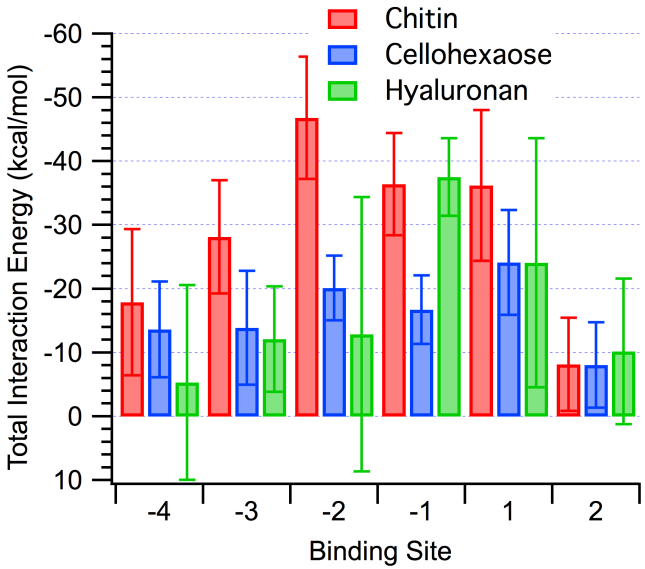
Exit the binding cleft

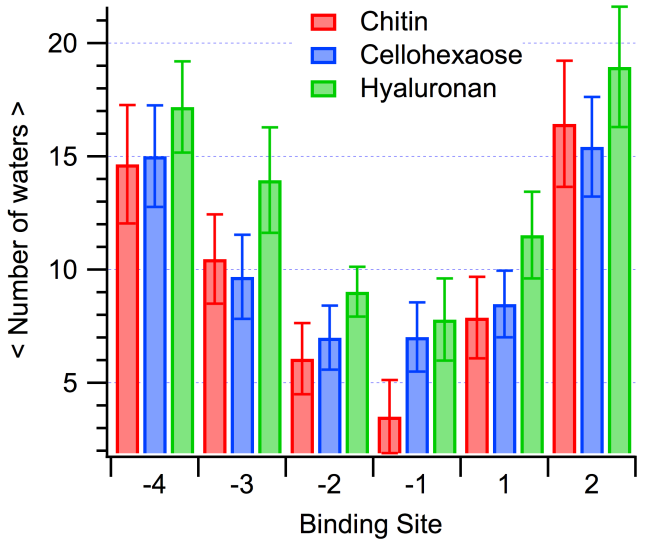
Hyaluronan : Potential Physiological Ligand ?

Analysis of polysaccharide binding dynamics



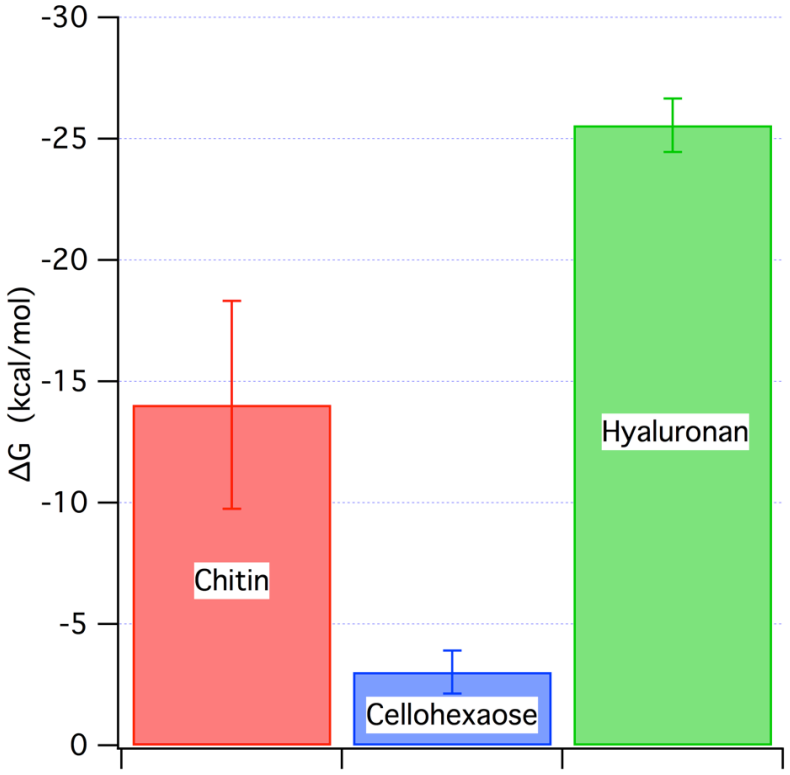






Hyaluronan : Potential Physiological Ligand ?

Relative affinity in terms of absolute binding free energy

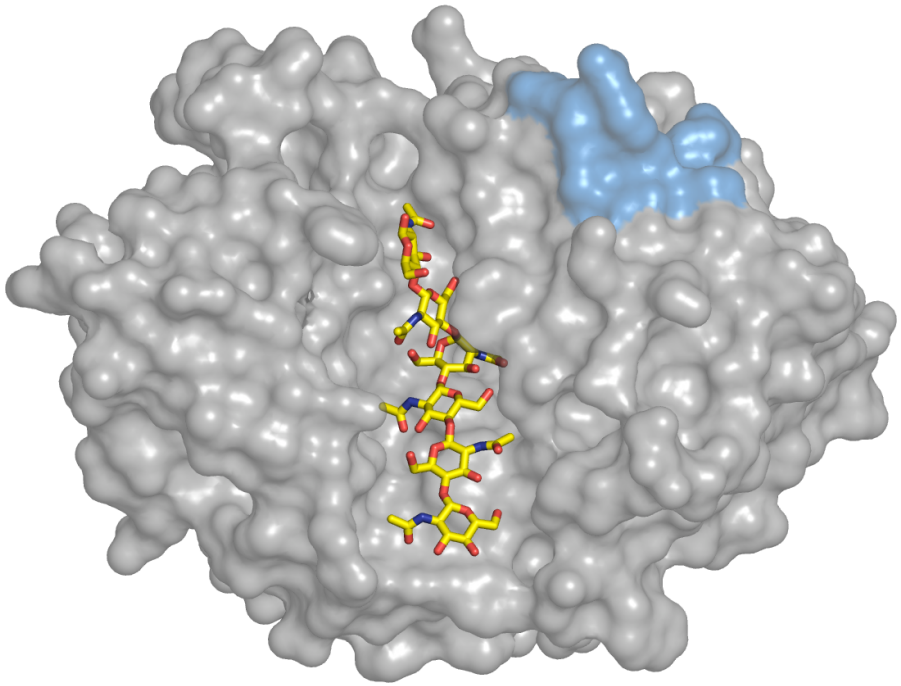


Absolute binding free energy of chitin, cellohexaose, and hyaluronan to YKL-40 calculated by FEP/λ-REMD method.

- Cellohexaose out of race due to low potential for enthalpic contribution.
- Chitin, being a native substrate, demonstrates ΔG resembling to that of family 18 chitinases.
- Hyaluronan shows most of the enthalpic contributions as from chitin, also supported by analysis of binding dynamics.
- Negative charge on hyaluronan adds to favorable change in ΔG through electrostatic contributions and solvation effects.

2

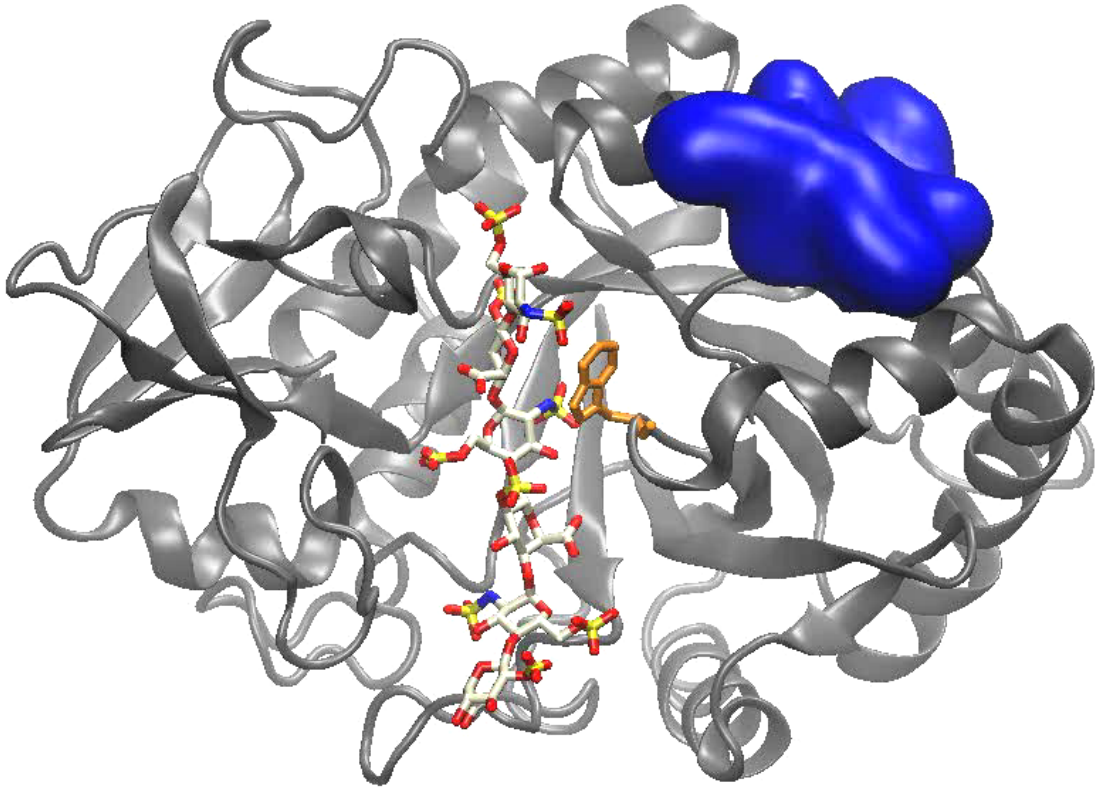
Putative Surface Binding Site for Heparin



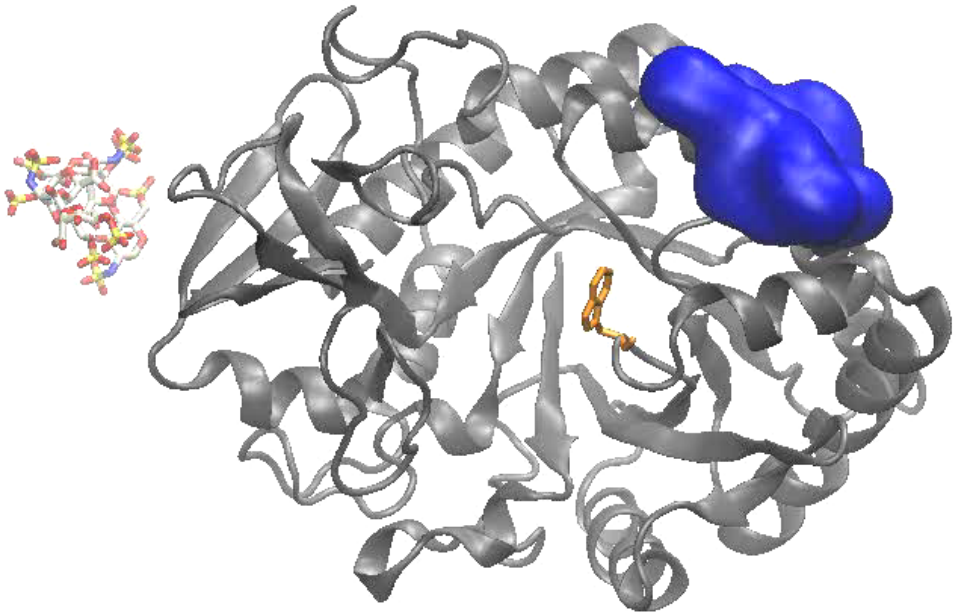
- Protein purification of YKL-40 uses its binding affinity for heparin
- Heparin – Highly sulfated carbohydrate and is part of Extra Cellular Matrix (ECM).
- No structural evidence of Heparin bound at the binding site.
- Complimentary features of heparin and protein surface?

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

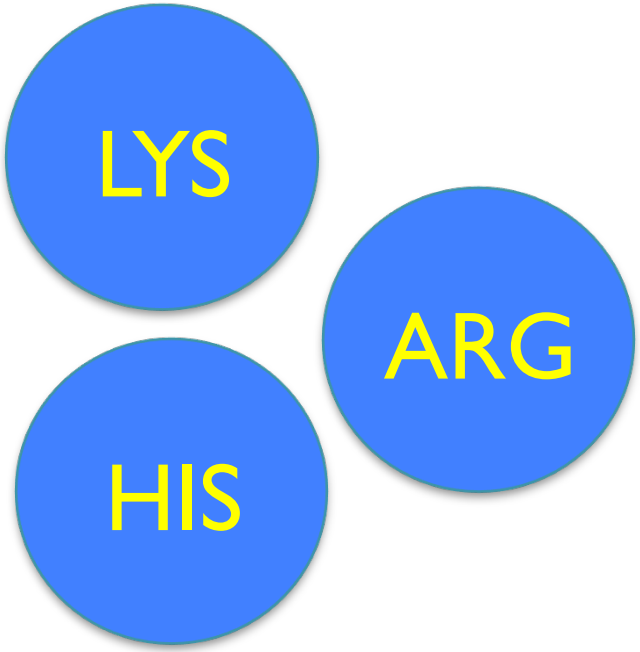
What Happens To Heparin?

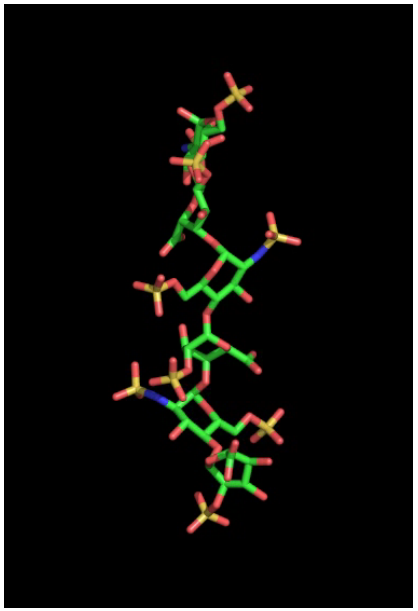


Starting From Different Coordinates!



What Amino Acids Comprise This Site?





Solution structure of Heparin

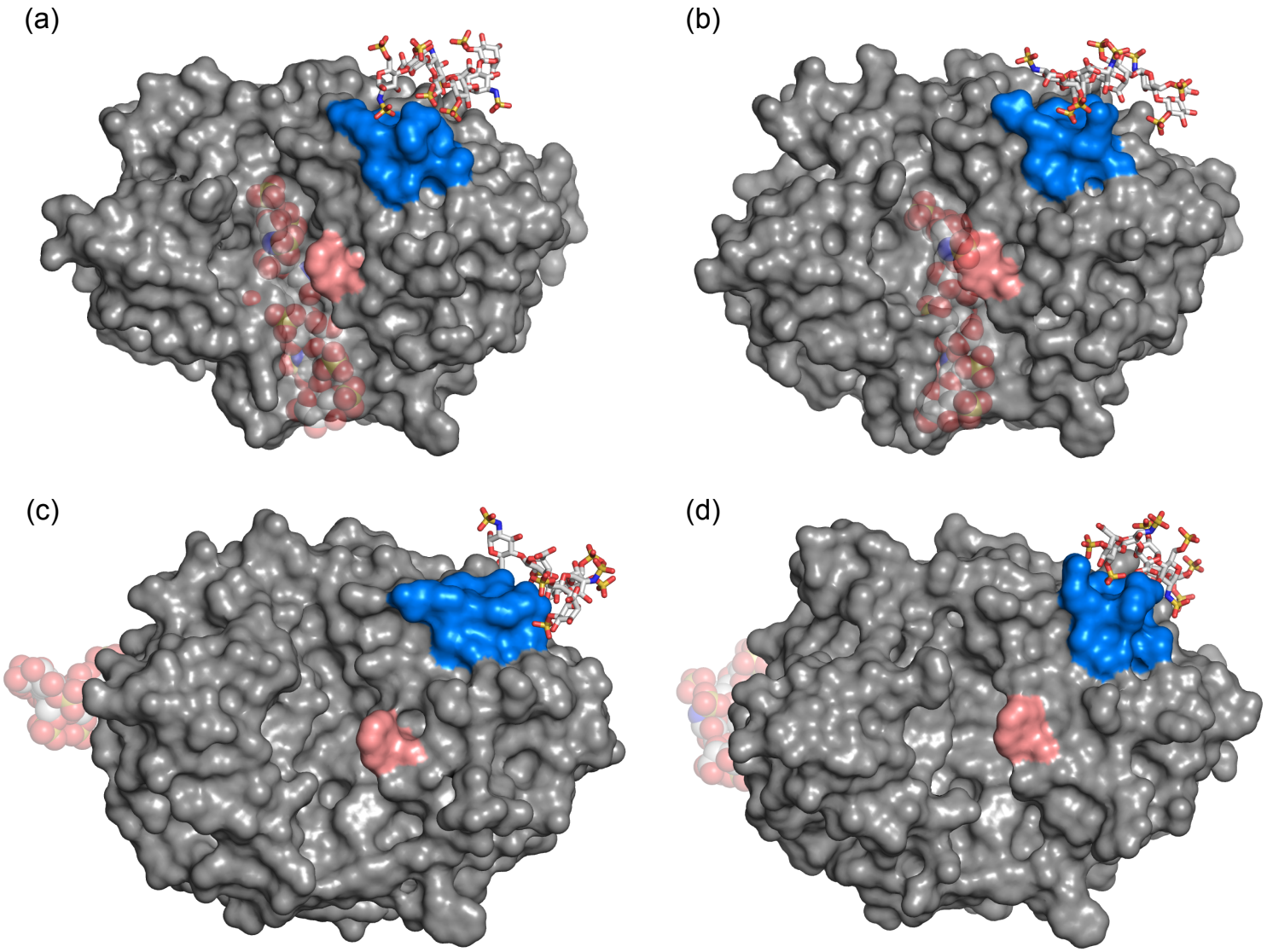
- Comparison with heparin-binding consensus sequences

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.

Cardin and Weintraub, Arteriosclerosis (1989)

Heparin Binding : Specific or Non-specific ?



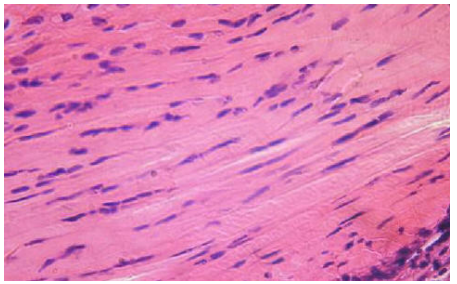
Heparin (white stick representation)

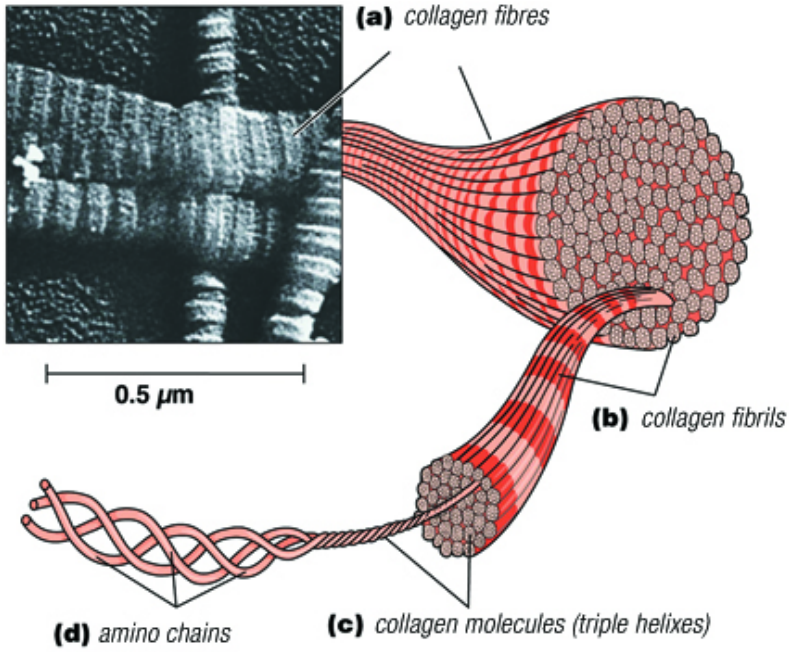
Putative heparin-binding site of YKL-40 (blue surface representation)

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.

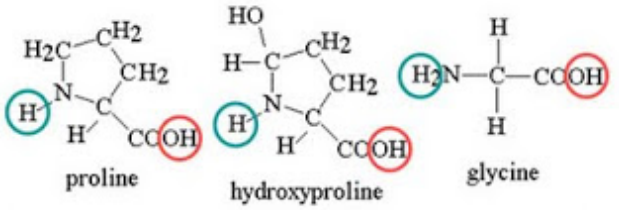




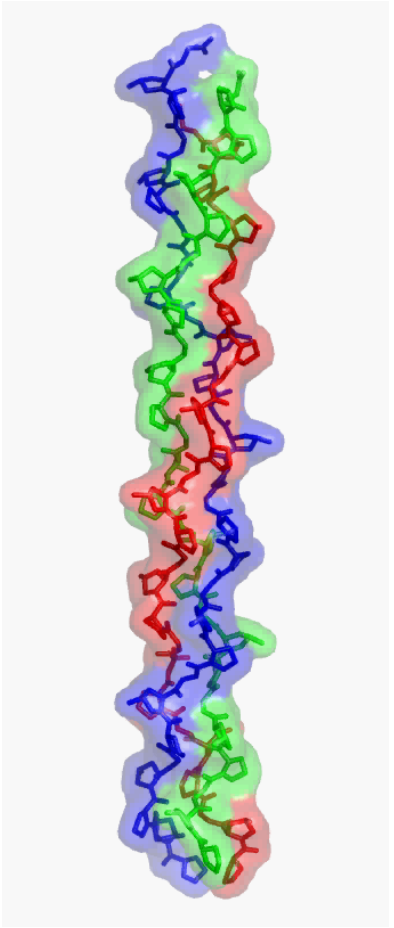
Bigg et al., J. Bio. Chem. (2006)

Images: 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 helical symmetries
- Four collagen peptides selected to represent helical and amino acid variability



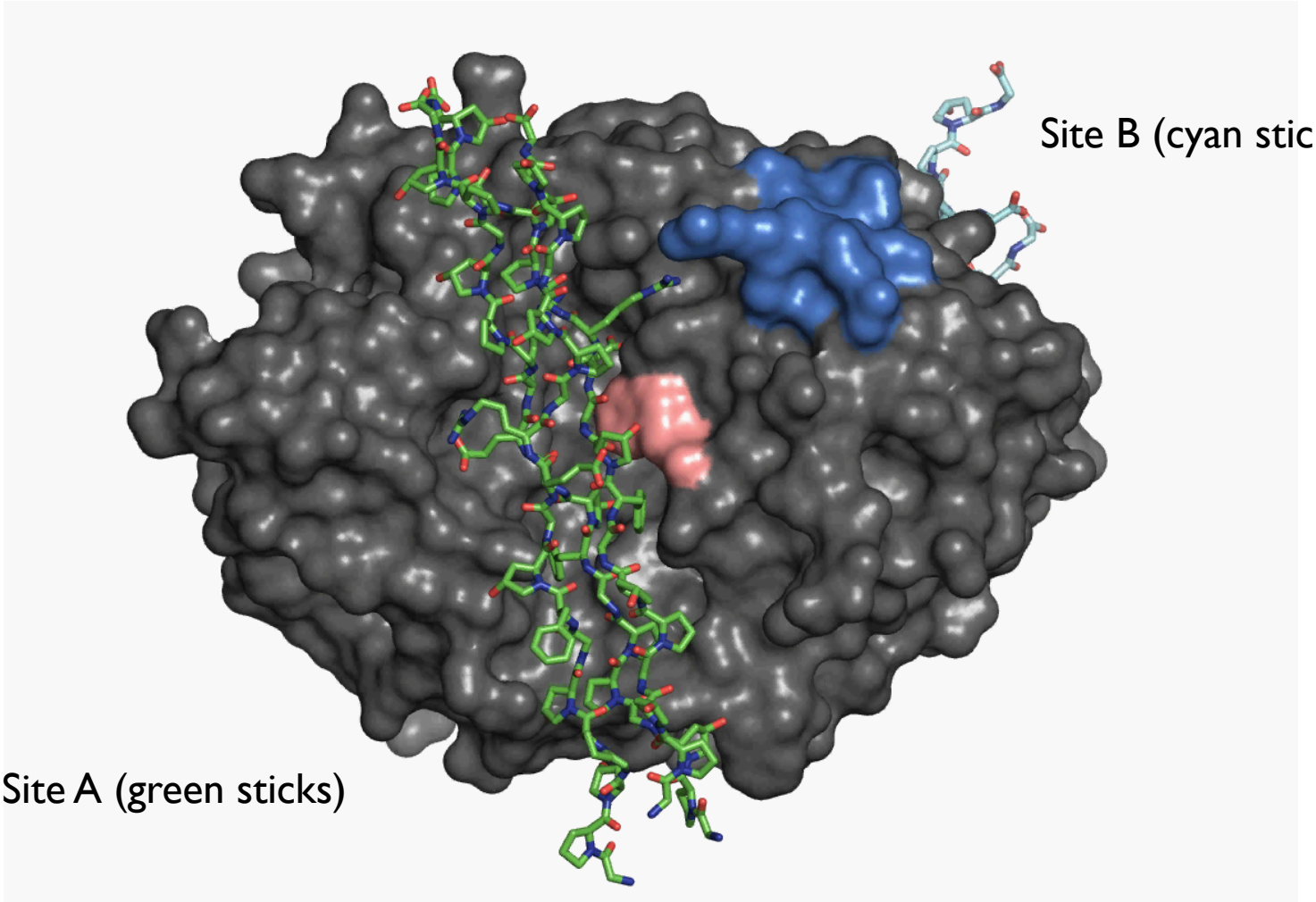
PDB ID : 1CAG

Collagen: Representative Peptide Models

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

Hydroxyproline

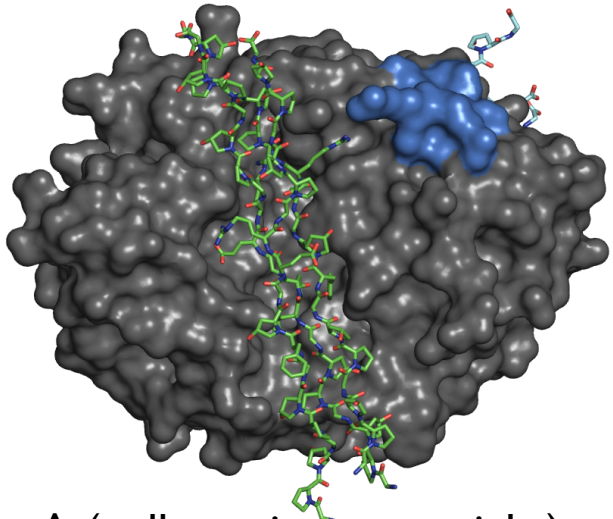
Where Is The Collagen Binding Site?



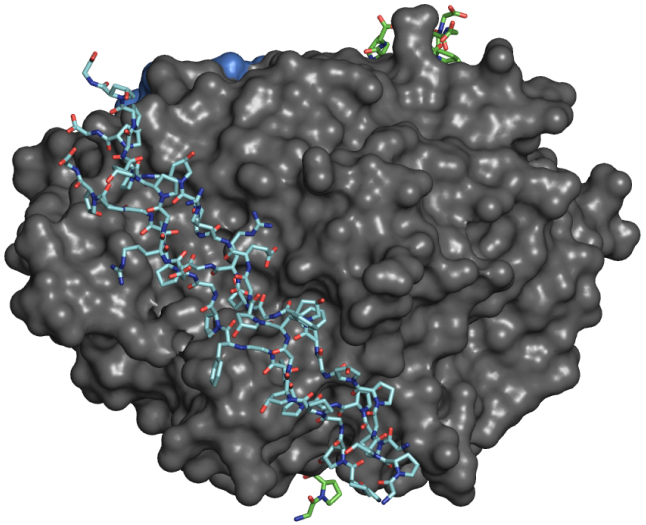
Molecular shape complementarity docking calculations predict collagen-like peptides can bind to YKL-40 in TWO possible orientations.

PatchDock - http://bioinfo3d.cs.tau.ac.il/PatchDock/

Binding Dynamics at Site A and Site B

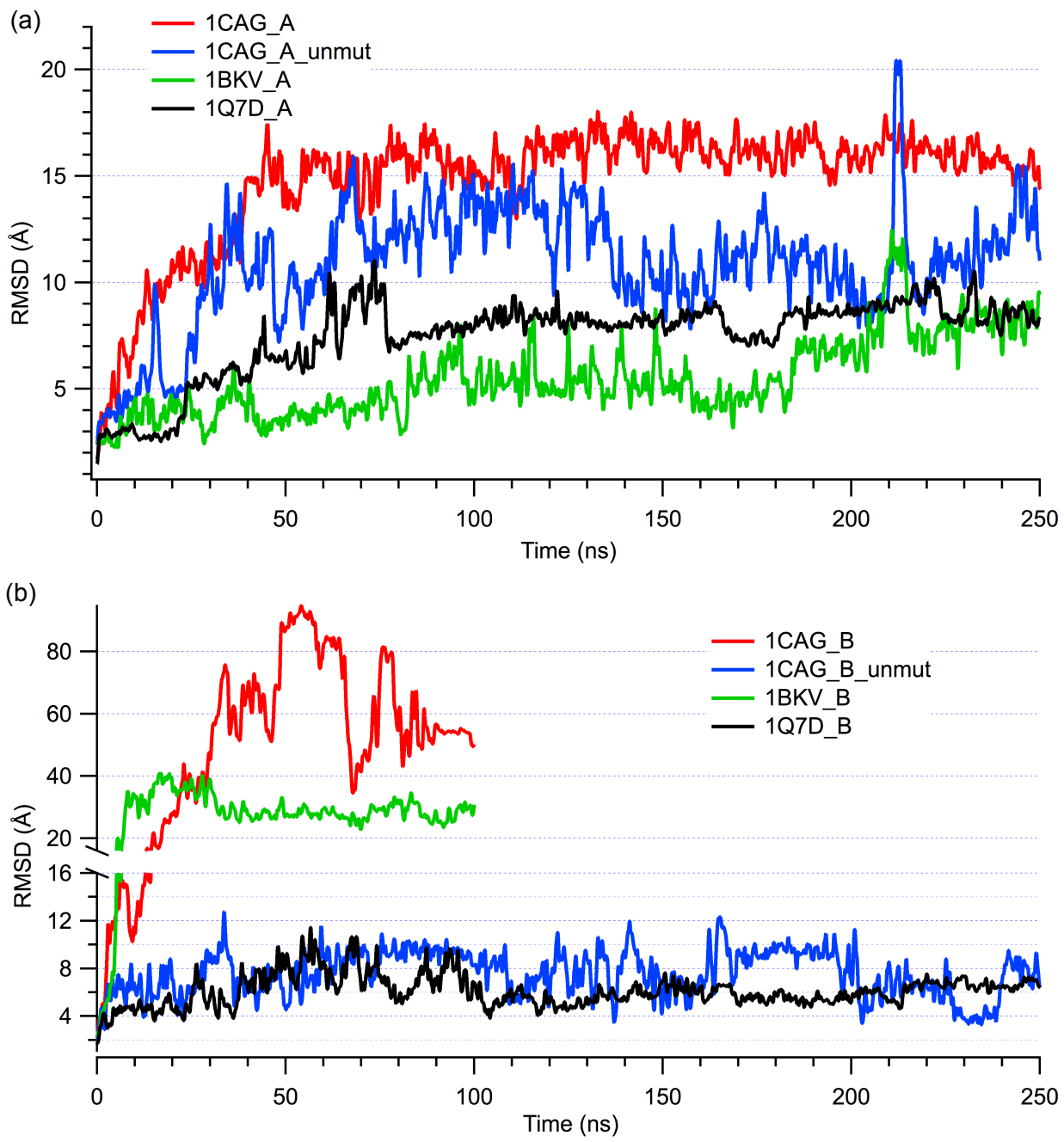


Site A (collagen in green sticks)

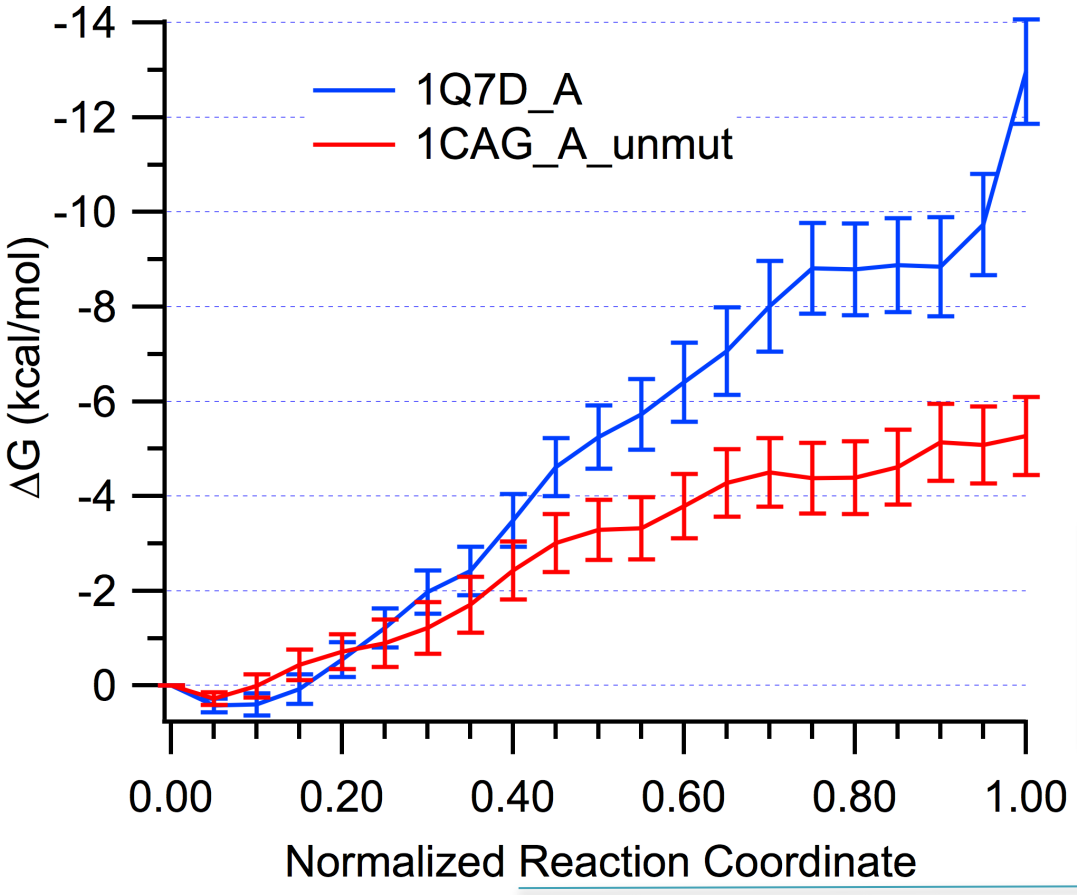


Site B (collagen in cyan sticks)

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

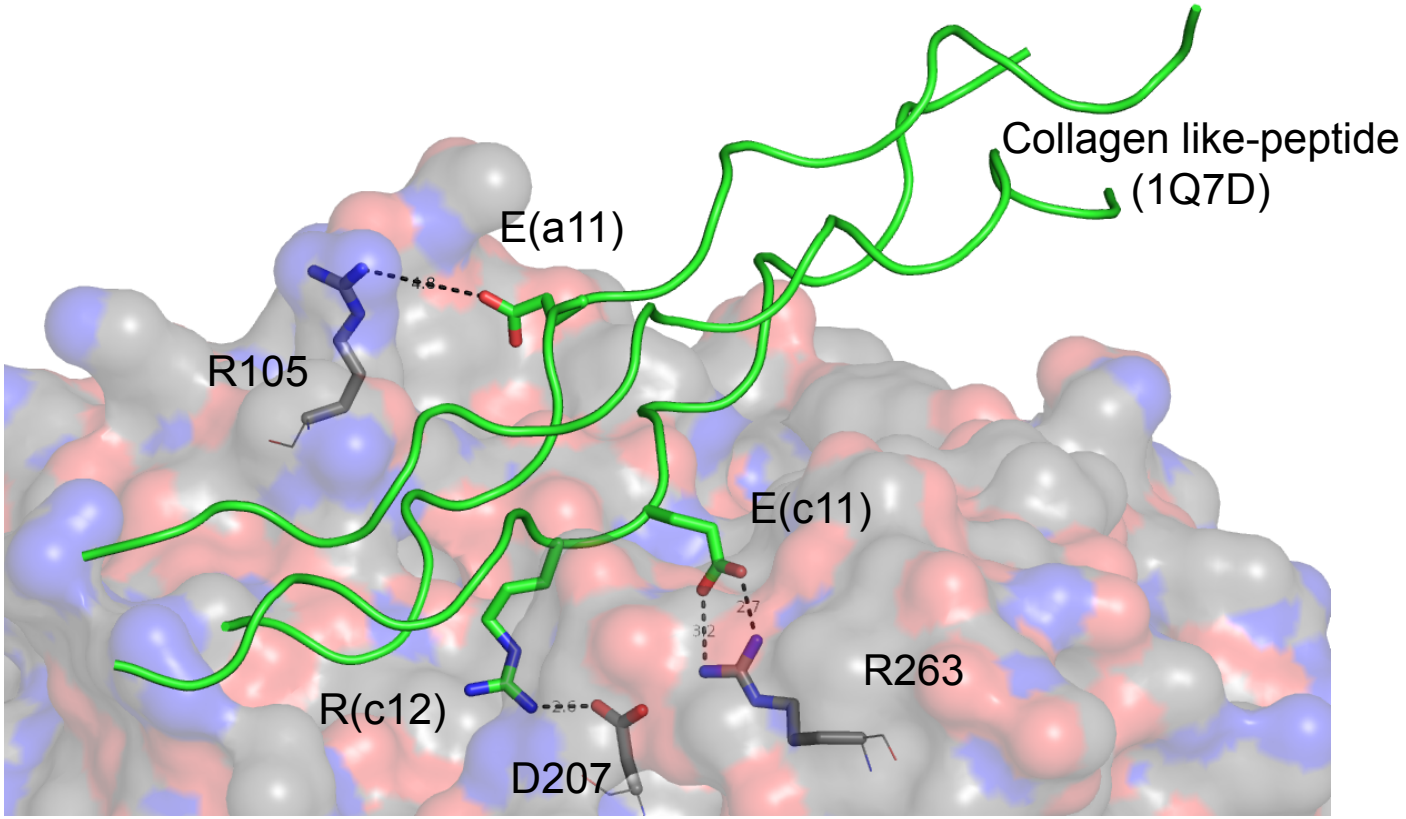

(a)
1CAG_A
1CAG_A_unmut
1BKV_A
1Q7D_A
RMSD (Å)
Time (ns)
(b)
1CAG_B
1CAG_B_unmut
1BKV_B
1Q7D_B
RMSD (Å)
Time (ns)

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



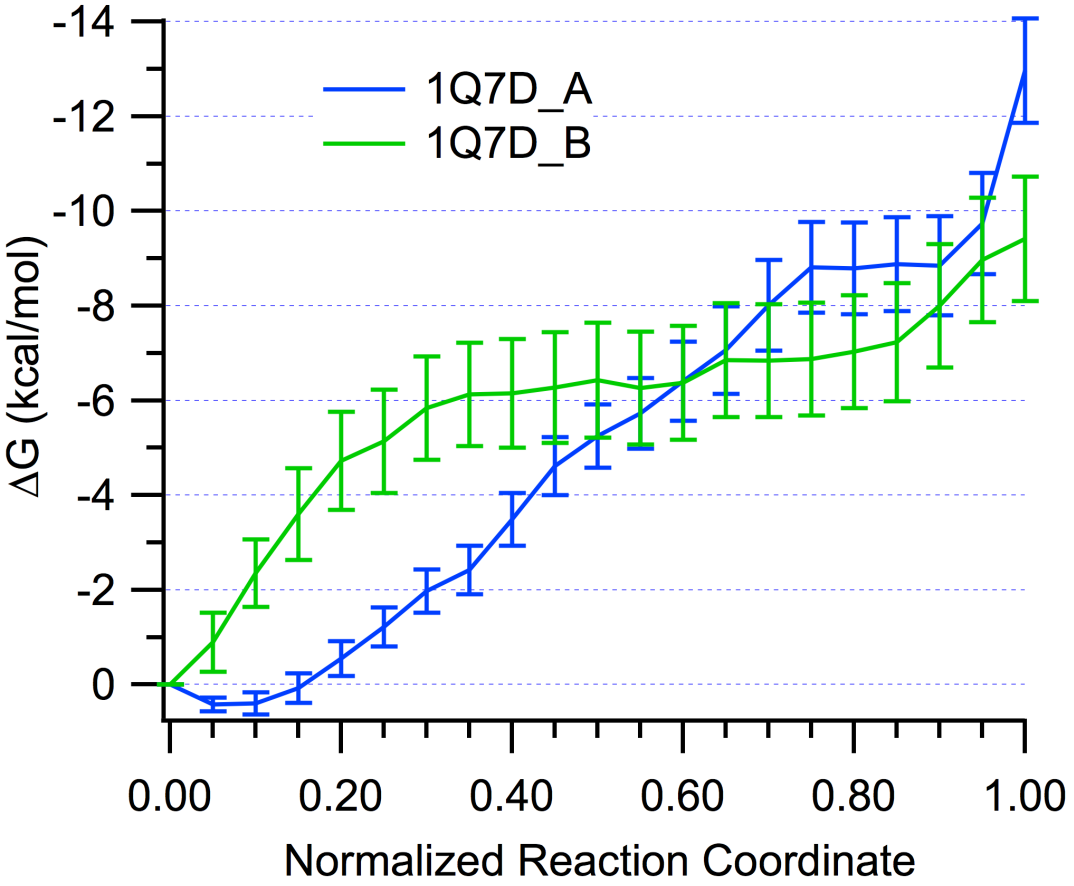
Potential of mean force (PMF) obtained from umbrella sampling MD simulations of the YKL-40-collagen system.

Collagen Binding site A



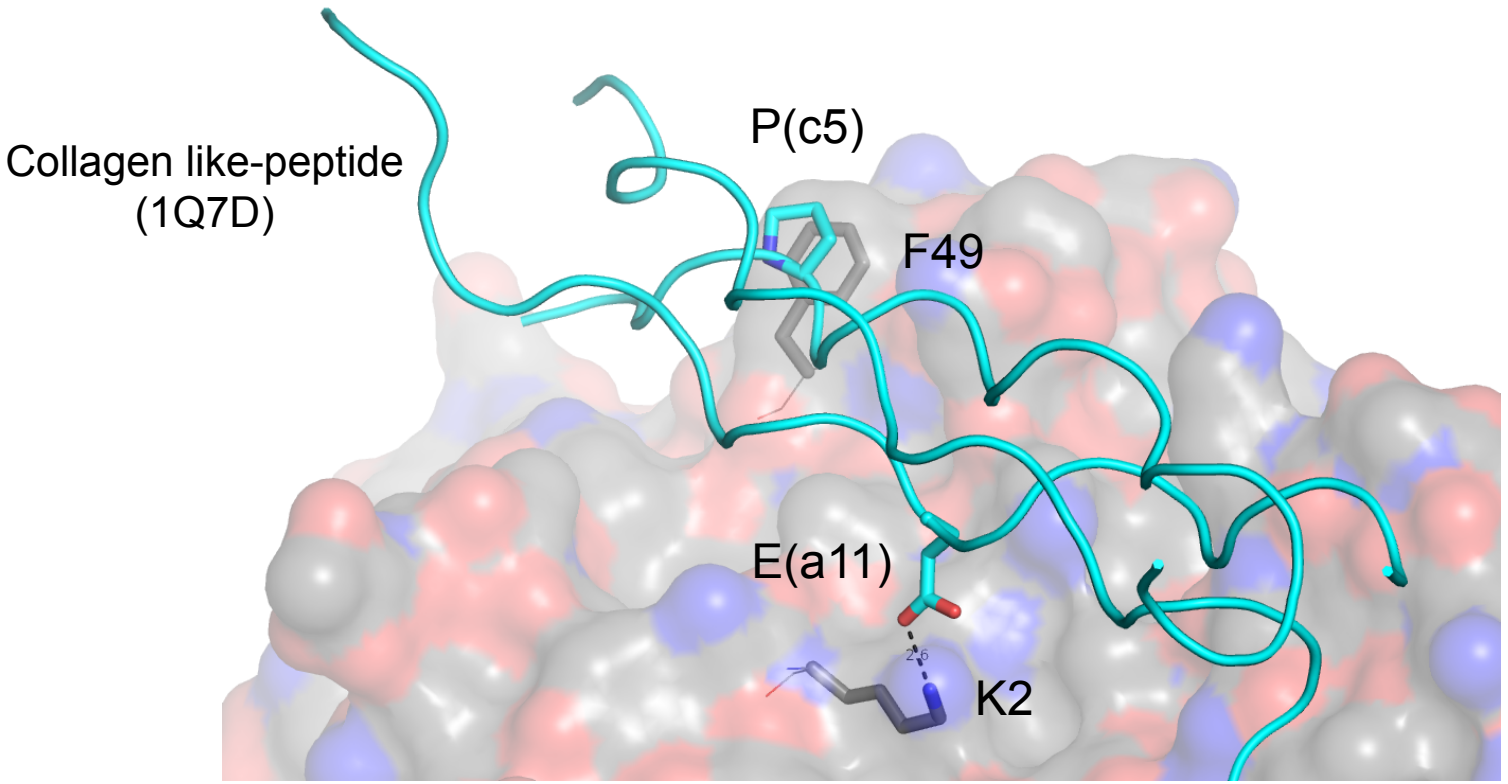
Binding Site A

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

Native Contacts for Umbrella Sampling

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

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

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

Conclusions from YKL-40 work…

- 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 insights towards understanding the functions of YKL-40 in mammalian cells.

Manuscript in final stages…

To be submitted to: *PLOS Computational Biology*

Inhibition of Mammalian Glycoprotein YKL-40: Identification of the Physiological Ligand

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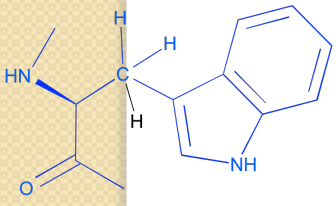
Abstract

YKL-40 is a non-catalytic mammalian glycoprotein and known biomarker associated with progression, severity, and prognosis of chronic inflammatory diseases and a multitude of cancers. Despite this well-documented association, conclusive identification of the lectin's physiological ligand, and accordingly biological function, has proven experimentally difficult. From experiments, YKL-40 has been known to bind chito-oligosaccharides; however, the natural presence of chitin in the human body has not yet been documented. Possible alternative ligands include proteoglycans, polysaccharides, and fibers such as collagen, all of which make up the mesh comprising the extracellular matrix. It is likely that YKL-40 is interacting with these additional polysaccharides or proteins within the body extending its function to cell biological roles such as mediating cellular receptors and cell adhesion and migration. Here, we consider polysaccharides including cello-oligosaccharides, hyaluronan, heparan sulfate, heparin, and chondroitin sulfate, as well as collagen-like peptides as potential physiological ligands for YKL-40. Molecular dynamics (MD) simulations resolve the molecular-level recognition mechanisms, as several of these potential ligands appear to bind YKL-40 in modes analogous to chito-oligosaccharides. Further, we calculate the free energy of binding the hypothesized ligands to YKL-40 to address the thermodynamic preference relative to chito-oligosaccharides. Our results suggest that chitohexaose and hyaluronan preferentially bind to YKL-40 over collagen, and hyaluronan is likely the preferred physiological ligand. The electrostatic interactions of hyaluronan with YKL-40 enhance the affinity of this ligand for YKL-40 over chitohexaose. Collagen binds in two locations at the surface of YKL-40 and may be related to its role in fibrillar formation. Finally, heparin non-specifically binds at the surface of YKL-40 as predicted from structural studies. Overall, YKL-40 likely binds to many natural ligands *in vivo*, but its concurrence with physical maladies may be related to the associated increase in hyaluronan. As a potential therapeutic target, these fundamental insights enable the rational design of YKL-40 antagonists to inhibit this action.

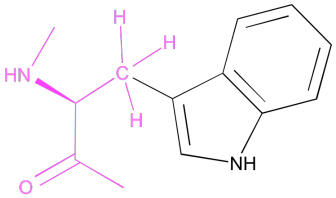
Themodynamic Integration and Molecular Modeling

- Thermodynamic integration (dual topology methodology)
  - Setup a hybrid system (containing both the wild-type and mutant residues)
  - Scale the interactions of the "product" and the "reactant" by λ
  - Separately decouple Electrostatic and Van der Waals interactions

Reactant (Trp)

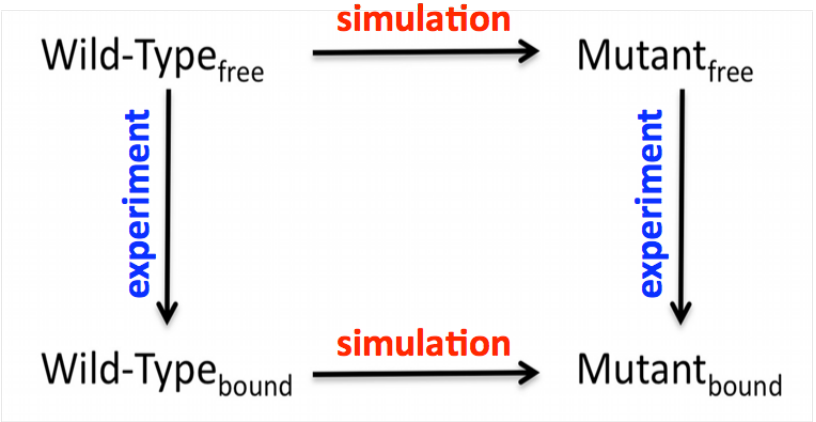


Product (Ala)



$$H(\lambda) = \lambda^n H_B + (1-\lambda)^n H_A$$

$$\Delta G = \int_0^1 \left\langle \frac{\partial H}{\partial \lambda} \right\rangle_\lambda d\lambda$$



Ligand Binding Free Energy Calculation

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

$$CBM * Ligand_{(solv)} \xrightarrow{\Delta G_1} CBM_{(solv)} + Ligand_{(vac)}$$

$$Ligand_{(solv)} \xrightarrow{\Delta G_2} Ligand_{(vac)}$$

$$CBM_{(solv)} + Ligand_{(solv)} \xrightarrow{\Delta G_b} CBM * Ligand_{(solv)}$$

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

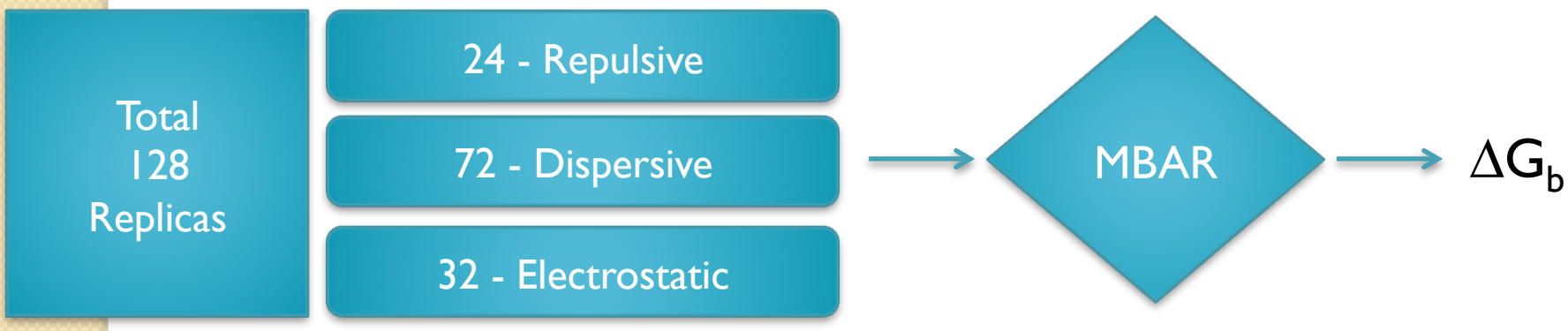
FEP/ λ -REMD

- Free Energy Perturbation

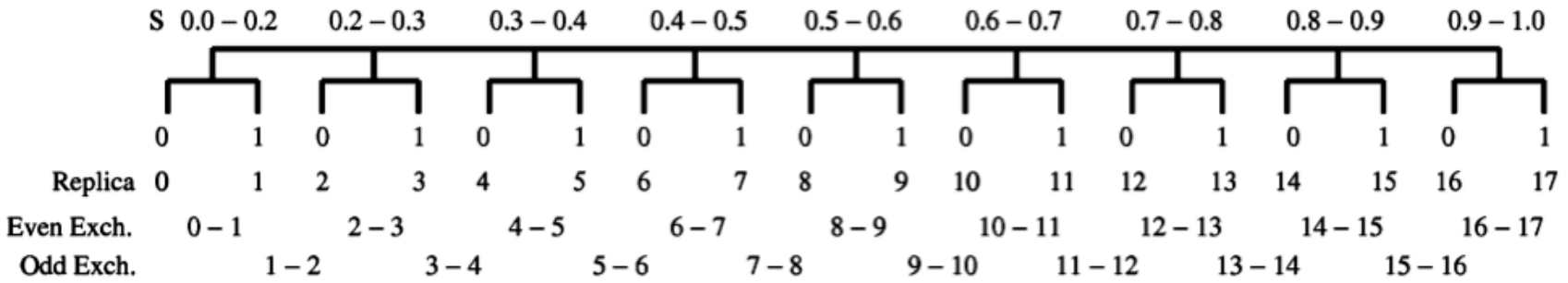
$$\Delta G(A \rightarrow B) = G_B - G_A = -k_B T \ln \langle \exp\left(-\frac{E_B - E_A}{k_B T}\right) \rangle_A$$

- Replica Exchange Molecular Dynamics

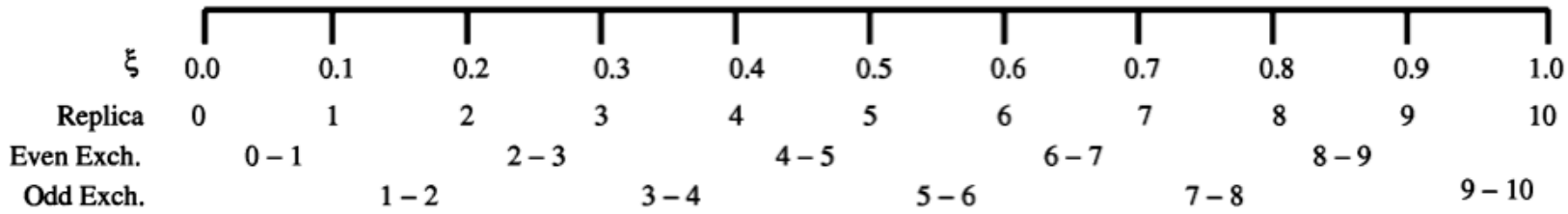
$$U = U_o + \lambda_{rep} U_{rep} + \lambda_{dis} U_{dis} + \lambda_{elec} U_{elec} + \lambda_{rstr} U_{rstr}$$



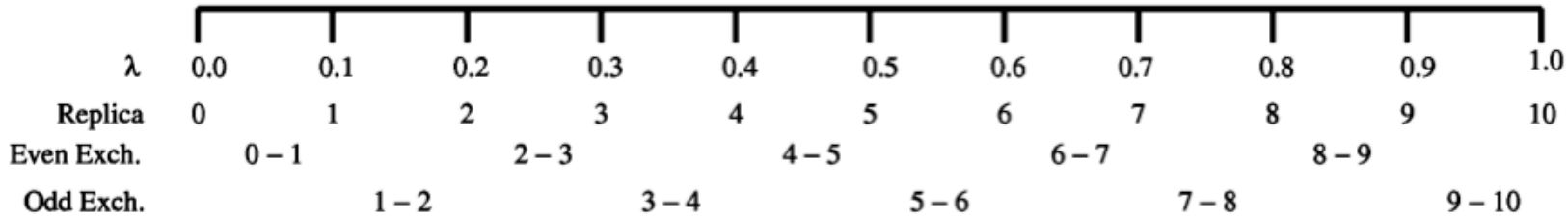
Jiang et al., *J. Chem. Theory Comput.*, 2009



(a) Switching–on of shifted LJ–WCA repulsive interaction

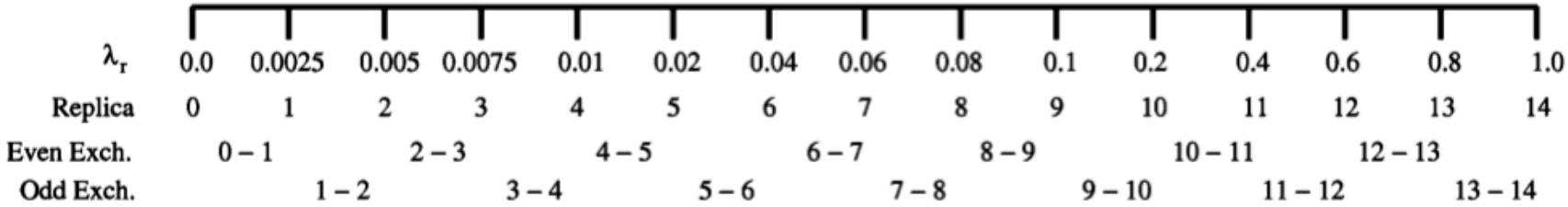


(b) Switching–on of LJ–WCA dispersive interaction



(c) Switching–on of electrostatic interaction

Jiang et al., *J. Chem. Theory Comput.*, 2009



(d) Removal of restraining potential

Functions of Non-bonded Interactions

shifted Weeks-Chandler-Anderson (WCA) repulsive and dispersive components of the Lennard- Jones potential

$$u_{ij}^{\mathrm{rep}}(r) = \begin{cases} \epsilon_{ij}\left[\left(\frac{R_{ij}^*}{r}\right)^{12} - 2\left(\frac{R_{ij}^*}{r}\right)^{6} + 1\right] & r \le R_{ij}^* \\ 0 & r > R_{ij}^* \end{cases}$$

$$u_{ij}^{\mathrm{dis}}(r) = \begin{cases} -\epsilon_{ij} & r \le R_{ij}^* \\ \epsilon_{ij}\left[\left(\frac{R_{ij}^*}{r}\right)^{12} - 2\left(\frac{R_{ij}^*}{r}\right)^{6}\right] & r > R_{ij}^* \end{cases}$$

$$v^{\mathrm{Coulomb}}(r) = \frac{Q_1 Q_2}{4\pi\epsilon_0 r},$$

Deng and Roux, *J. Phys. Chem. B,* 2004

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Thank You…

Questions?