MEDC 691– Glycochemistry and Glycobiology
VCU

Thermodynamics, kinetics, and dynamics of protein-GAG complexes

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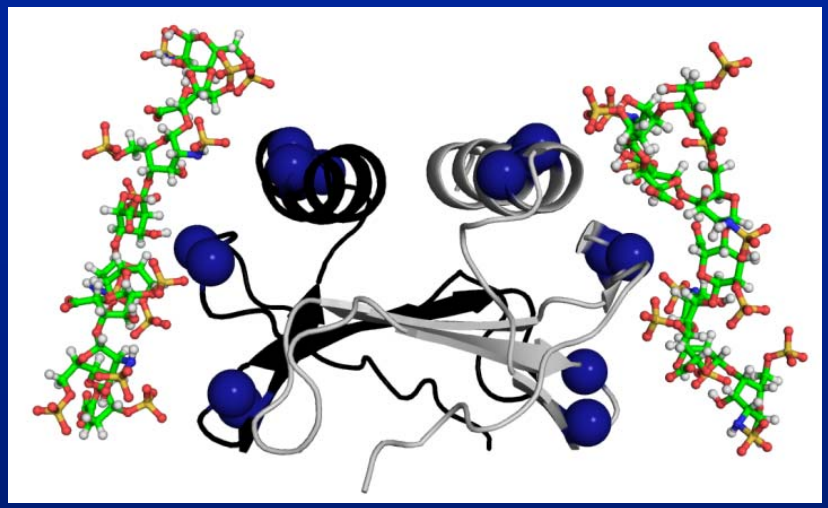
utmb Health



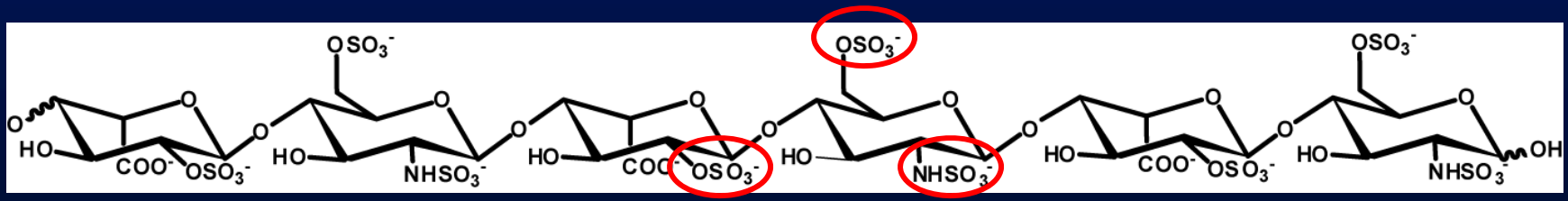
A complete understanding of the molecular basis of protein-GAG interactions require the relationship between structure, dynamics, kinetics, and thermodynamics

Why is it important?

Glycosaminoglycan (GAG) Binding and Function

heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate

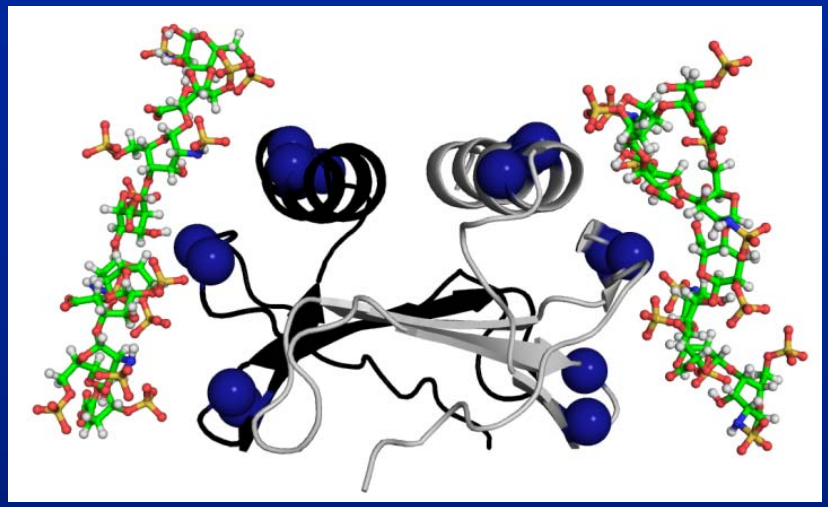
- ✓ Linear polysaccharide chains linked to Ser of proteoglycan
- ✓ Repeating disaccharide unit; Heterogeneous N- and O-sulfation, N-acetylation
- ✓ GAG binding is essential for formation of chemotactic gradient.
- ✓ GAG binding modulates chemokine monomer-dimer equilibrium



What are the most pressing questions that limit our understanding of how GAGs bind proteins?

Knowledge of the molecular mechanisms and the structural basis are lacking

- binding affinities, stoichiometry, kinetics, and thermodynamics
- structural features such as monomer vs. dimer, identification of GAG-binding residues and their roles in determining affinity, specificity, geometry, etc.



How do we gain insights into the structure, dynamics, kinetics, and thermodynamics of protein-GAG complexes.
What are the experimental and computational methods?
Why is this knowledge so hard to come by?

Why study and what is thermodynamics?

How does the structural features mediate binding
What is the binding mode?
Which residues and why these residues?
What is the binding affinity – what is the contribution of the individual residues?
Are the binding interactions additive or cooperative?
What is the relationship between kinetics and thermodynamics?
Why kinetics are important for understanding function?
Relationship between kinetics and thermodynamics?
Relationship between dynamics and thermodynamics?

challenges/factors that need to be considered for studying thermodynamics of protein-GAG interactions

- ✓ Knowledge of the structures
- ✓ Limitations of the biophysical/structural techniques
- ✓ Complexity of the binding interactions
- ✓ Complexity of the naturally occurring GAGs
- ✓ non-availability of ‘homogeneous’ GAGs

challenges/factors that need to be considered for studying thermodynamics of protein-GAG interactions

specificity vs. promiscuity - why is it so important

the same GAG (say HS) binds multiple proteins; therefore, proteins must have evolved to bind GAG with the objective of eliciting the required response.

What about diversity of HS sequences?

in other words, what interactions mediate binding?

- Electrostatic vs. non-electrostatic interactions?
- Role of Lys, Arg, and His – what are the unique characteristics of these charged amino acids? – is there a preference for one over the other?

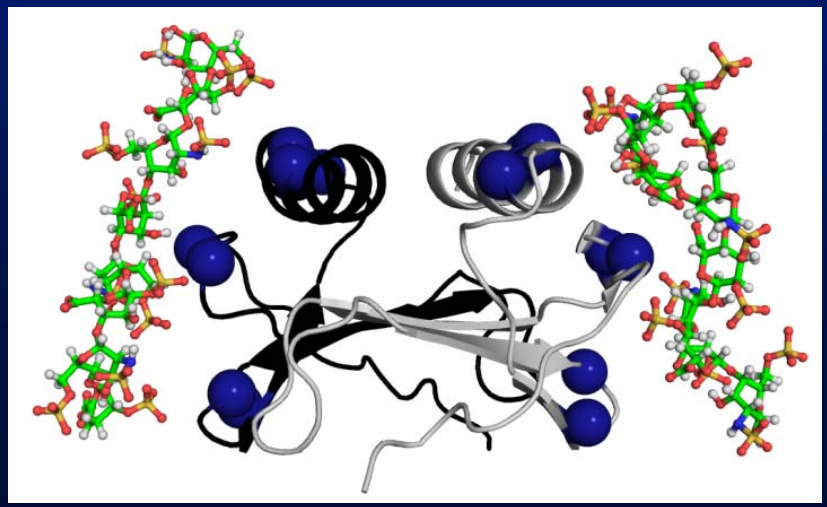
Why study thermodynamics

$$\Delta G = \Delta H - T\Delta S$$

$$\Delta G = -RT\ln K$$

How do you interpret enthalpy and entropy contributions

Availability of structures and/or models can provide residue-specific interactions.



$\Delta G = \Delta H - T\Delta S$

$\Delta G = -RT\ln K$

Enthalpy of binding.

$\Delta G = \Delta H - T\Delta S$

Enthalpy can be interpreted in terms of favorable packing, ionic, and H-bonding interactions.

Structures are essential to describe the enthalpy of binding.

Enthalpy – order

$\Delta G = \Delta H - T\Delta S$

$\Delta G = -RTlnK$

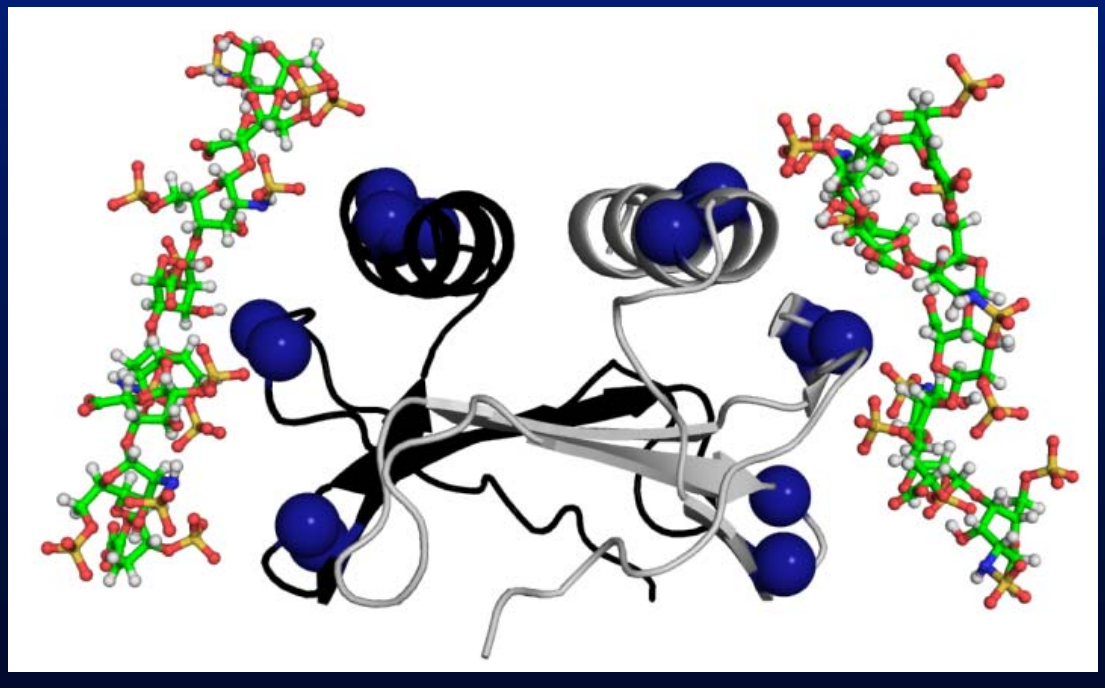
Change in dynamics in the protein and GAG (or increased disorder) -- backbone and side chain dynamics

Release and reorganization of water.
Release of cations such as Na^+

Structures are essential to describe the enthalpy of binding.
Entropy - disorder

Thermodynamics of protein-GAG interactions – what do we know, where do we stand?

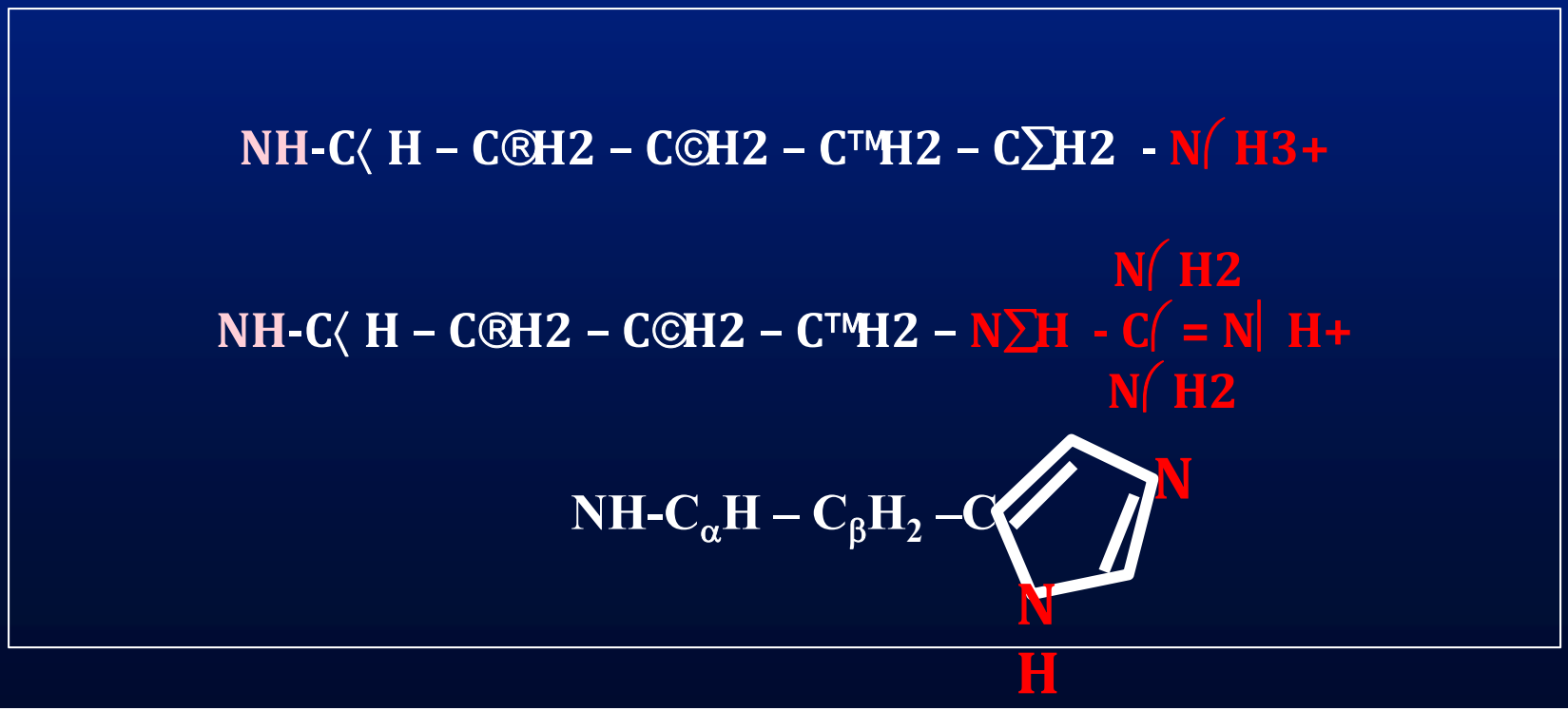
1) Knowledge of the structures are critical
2) Role of ionic interactions – electrostatics – short and long-range
3) Role of non-ionic interactions – H-bonding, polar, hydrophobic



Challenges and Progress

Role of Lys, Arg, and His – what are the unique characteristics of these charged amino acids? – is there a preference for one over the other?

Techniques to characterize ionic interactions – solution NMR and molecular dynamics (MD)



Challenges and Progress

- Role of polar residues– Asparagine, Glutamine, Serne what are the characteristics of these amino acids?
- Role of polar interactions – low energy interactions - Specificity vs. affinity?
- Techniques to characterize these interactions – solution NMR and molecular dynamics (MD)

NH-C⟨ H – C®H2 – CO - NH2
NH-C⟨ H – C®H2 – C©H2 – CO - NH2
NH-C⟨ H – C®H2 – OH

Challenges and Progress

- Electrostatic and ionic interactions
- H-bonding interactions
- Hydrophobic/packing interactions
- Specificity vs. affinity?

What residues mediate what interactions – best studied (but still not well understood) are the ionic interactions.

GAGs are negatively charged and so the role of ionic interactions are intuitive. Mutational studies confirm the importance of ionic interactions.

What different techniques can tell us?

1) Techniques to measure binding affinities, stoichiometry, kinetics, and thermodynamics

2) Techniques to characterize structural features and molecular basis for binding (geometry, residues involved in binding).

- Fluorescence (anisotropy, intensity); CD
- ITC - thermodynamics
- SPR - kinetics
- NMR – residue-specific binding
- X-ray crystallography
- MD and modeling – residue-specific binding

What ITC (Isothermal Titration Calorimetry) can tell us?

In addition to binding affinity (ΔG), insights into the thermodynamics (ΔH, ΔS), heat capacity (ΔCp), and stoichiometry of binding can be measured in a straight forward manner from a single titration.

$\Delta G = \Delta H - T\Delta S$

$\Delta G = -RT\ln K$

Stoichiometry of binding – one of the few techniques which can give number of proteins bound per GAG.

Crucial to understand the relationship between stoichiometry, affinity, GAG size, differences between heparin and heparan sulfate structures (NS-NA-NS modular structure).

What ITC can tell us?

In addition to binding affinity, insights into the thermodynamics (enthalpy and entropy), heat capacity (ΔCp), and stoichiometry of binding can be measured in a straight forward manner from a single titration.

$\Delta G = \Delta H - T\Delta S$

$\Delta G = -RT\ln K$

Advantages –

- No modifications/labeling
- Measurements made in solution – no immobilization.
- Binding constants from nM to mM
- Under ideal conditions, ITC can also give insights into molecular mechanisms such as cooperativity.

Design of high affinity and high specificity inhibitors --- Why knowing enthalpy and entropy contributions important?

Can be exploited to maximize favorable interactions,
eliminate unfavorable interactions –
essential for designing high-affinity, high-specificity GAG decoys.

have to optimize both enthalpy and entropy of binding.
Entropic contribution to binding is computationally difficult to capture unless and until measurements such as ITC are carried out.
Most drug discovery approaches that involve docking drug molecules discount entropy and/or the scoring functions are woefully inadequate.

High through-put ITC in drug discovery – second/third level screening – will be a boon for convergence and the design process.
Challenge – requires relatively large amounts of protein than traditional HTS approaches. Technique is being automated, miniaturized, making it more approachable.

ITC: Nuts and Bolts

How do you get the thermodynamic parameters from ITC

$\Delta G = \Delta H - T\Delta S$; $\Delta G = -RT\ln K$

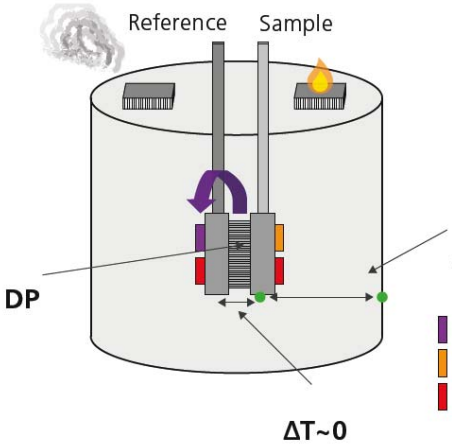
$$Q = \frac{nP_t \Delta H V_0}{2}\left[1 + \frac{X_t}{nP_t} + \frac{1}{nK_a P_t} - \sqrt{\left(1 + \frac{X_t}{nP_t} + \frac{1}{nK_a P_t}\right)^2 - \frac{4X_t}{nP_t}}\right] \quad (2)$$

$$\Delta Q_{(i)} = Q_{(i)} - \frac{dV_{(i)}}{V_0}\left[\frac{Q_{(i)} + Q_{(i-1)}}{2}\right] - Q_{(i-1)} \quad (3)$$

ITC instrumentation

Malvern microcalorimeter

How do they work?

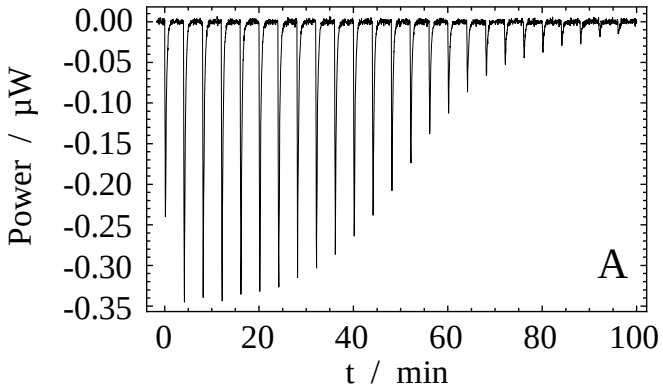


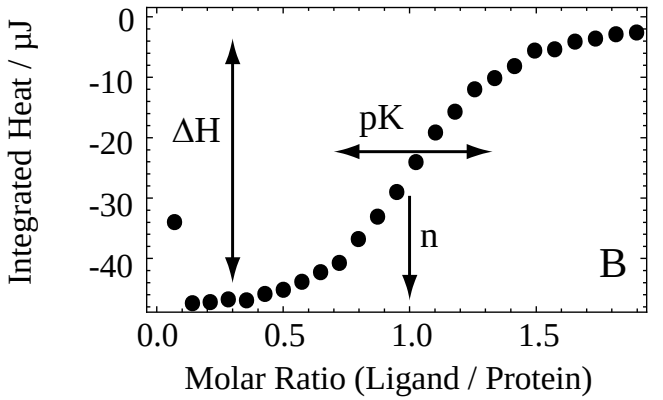
The DP is a measured power differential between the reference and sample cells to maintain a zero temperature between the cells

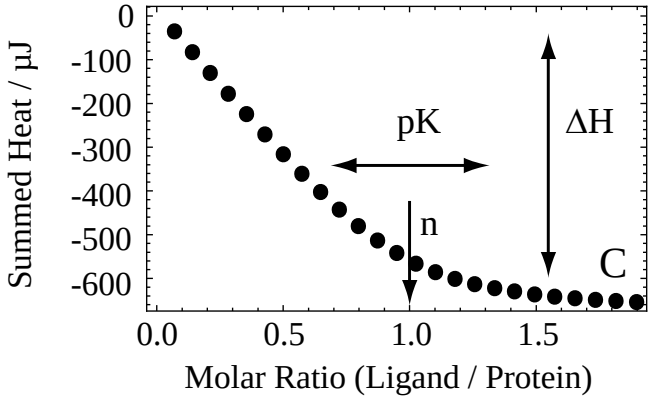
- Reference Calibration Heater
- Sample Calibration Heater
- Cell Main Heater

DP - Differential Power
ΔT - Temperature difference

What do you do in an ITC experiment







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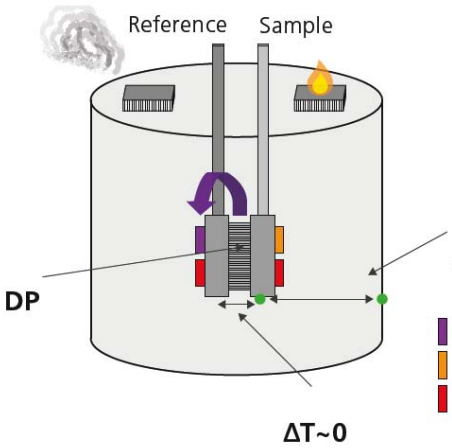

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- Reference Calibration Heater
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DP - Differential Power

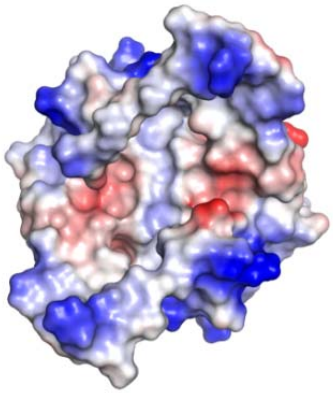
ΔT - Temperature difference

Disadvantages

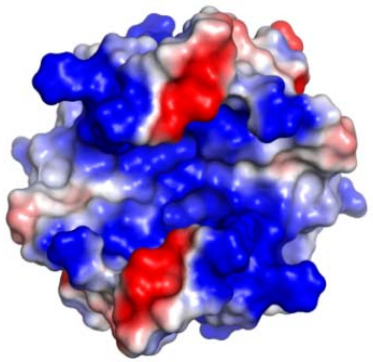
1) Large levels of protein and GAGs are required
2) sufficient heat release (observable) is necessary.
3) requires some expertise on the operator
4) precipitation could occur during the titration.

```
                    B1          B2 B3                                  B4  B5          B6/B7   B8
CXCL1       ASVATELRCQCLQTLQ-GIHPKNIQSVNVKSPGPHCAQTEVIATLKNGRKACLNPASPIVKKIIEKMLNSDKSN
CXCL5   AGPAAAVLRELRCVCLQTTQ-GVHPKMISNLQVFAIGPQCSKVEVVASLKNGKEICLDPEAPFLKKVIQKILDGGNKEN
CXCL7         AELRCMCIKTTS-GIHPKNIQSLEVIGKGTHCNQVEVIATLKDGRKICLDPDAPRIKKIVQKKLAGDESAD
CXCL8       SAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSDGRELCLDPKENWVQRVVEKFLKRAENS
CXCL2       APLATELRCQCLQTLQ-GIHLKNIQSVKVKSPGPHCAQTEVIATLKNGQKACLNPASPMVKKIIEKMLKNGKSN
CXCL3       ASVVTELRCQCLQTLQ-GIHLKNIQSVNVRSPGPHCAQTEVIATLKNGKKACLNPASPMVQKIIEKILNKGSTN
CXCL6     GPVSAVLTELRCTCLRVTLR-VNPKTIGKLQVFPAGPQCSKVEVVASLKNGKQVCLDPEAPFLKKVIQKILDSGNKKN
mKC         GAPIANELRCQCLQTM-AGIHLKNIQSLKVLPSGPHCTQTEVIATLKNGREACLDPEAPLVQKIVQKMLKGVPK
mMIP2         VVASELRCQCLKTLP-RVDFKNIQSLSVTPPGPHCAQTEVIATLKGGQKVCLDPEAPLVQKIIQKILNKGKAN
```

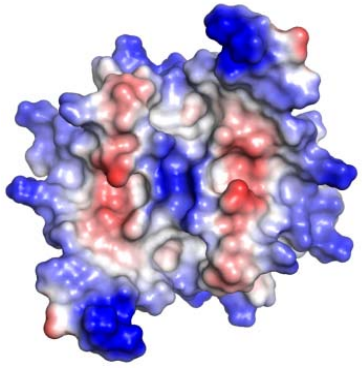
	B1	B2	B3	B4	B5	B6	B7	B8
CXCL1	Y	Y	Y	Y	Y	Y	Y	Y
CXCL5	N	Y	Y	Y	Y	Y	Y	Y
CXCL7	N	Y	Y	N	Y	N	Y	Y
CXCL8	N	Y	Y	N	Y	N	Y	Y



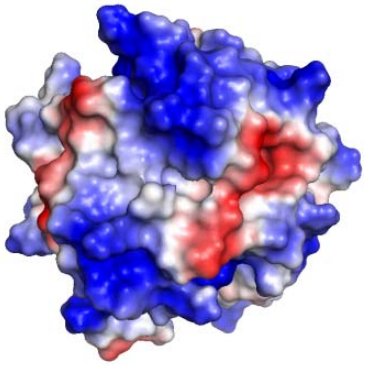
CXCL5



CXCL1

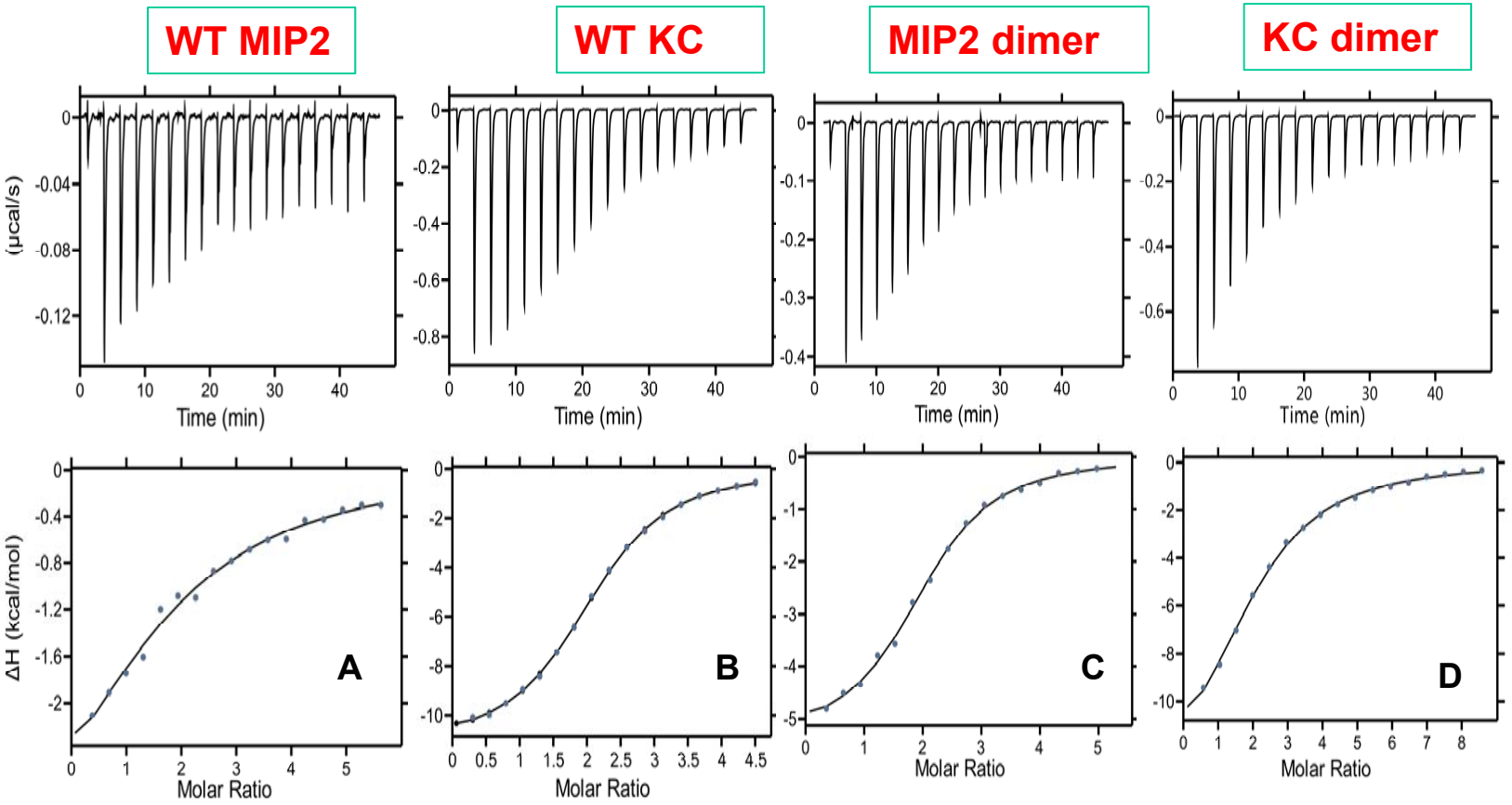


CXCL8



CXCL7

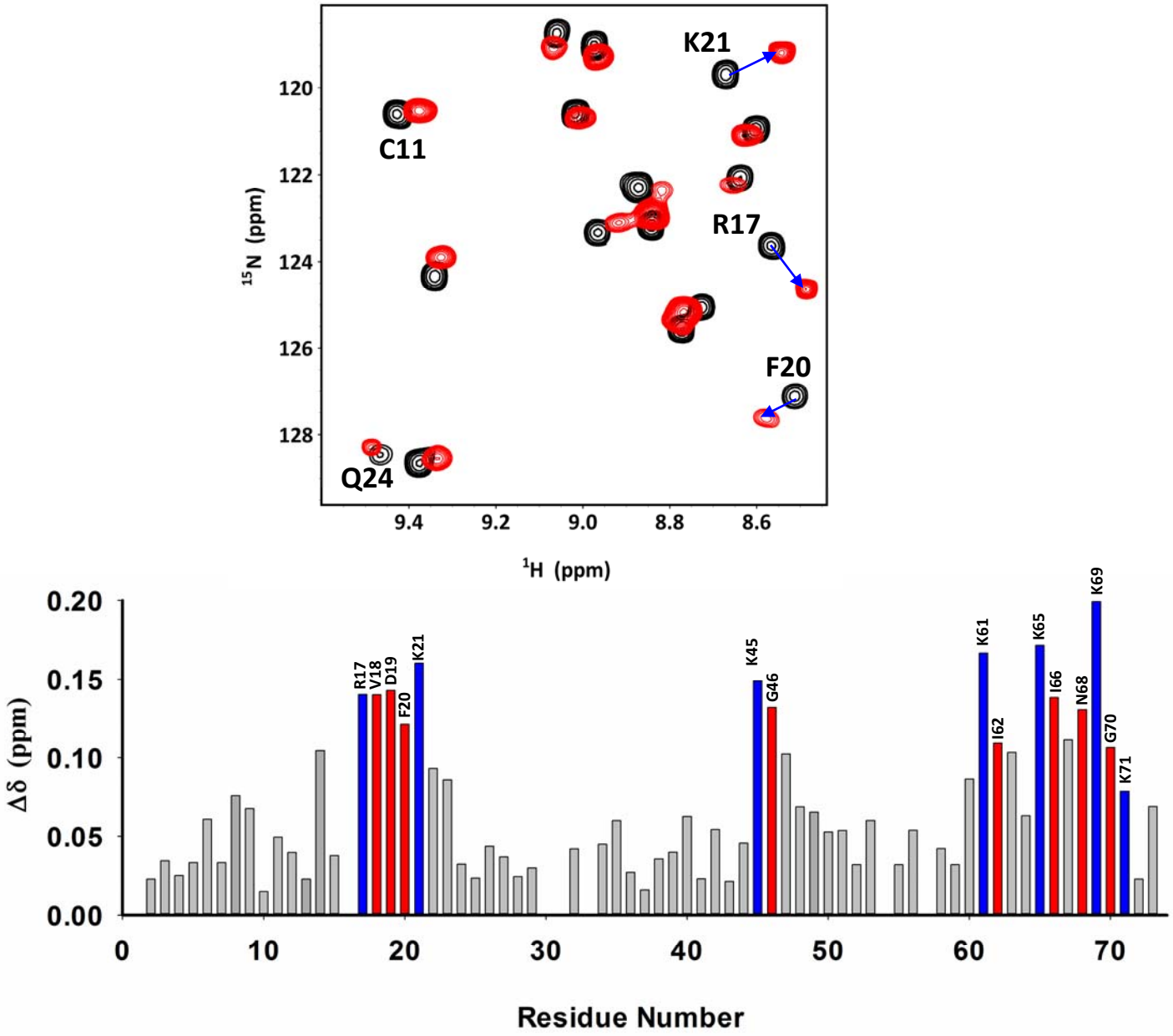
ITC profiles of heparin dp8 binding to KC and MIP2



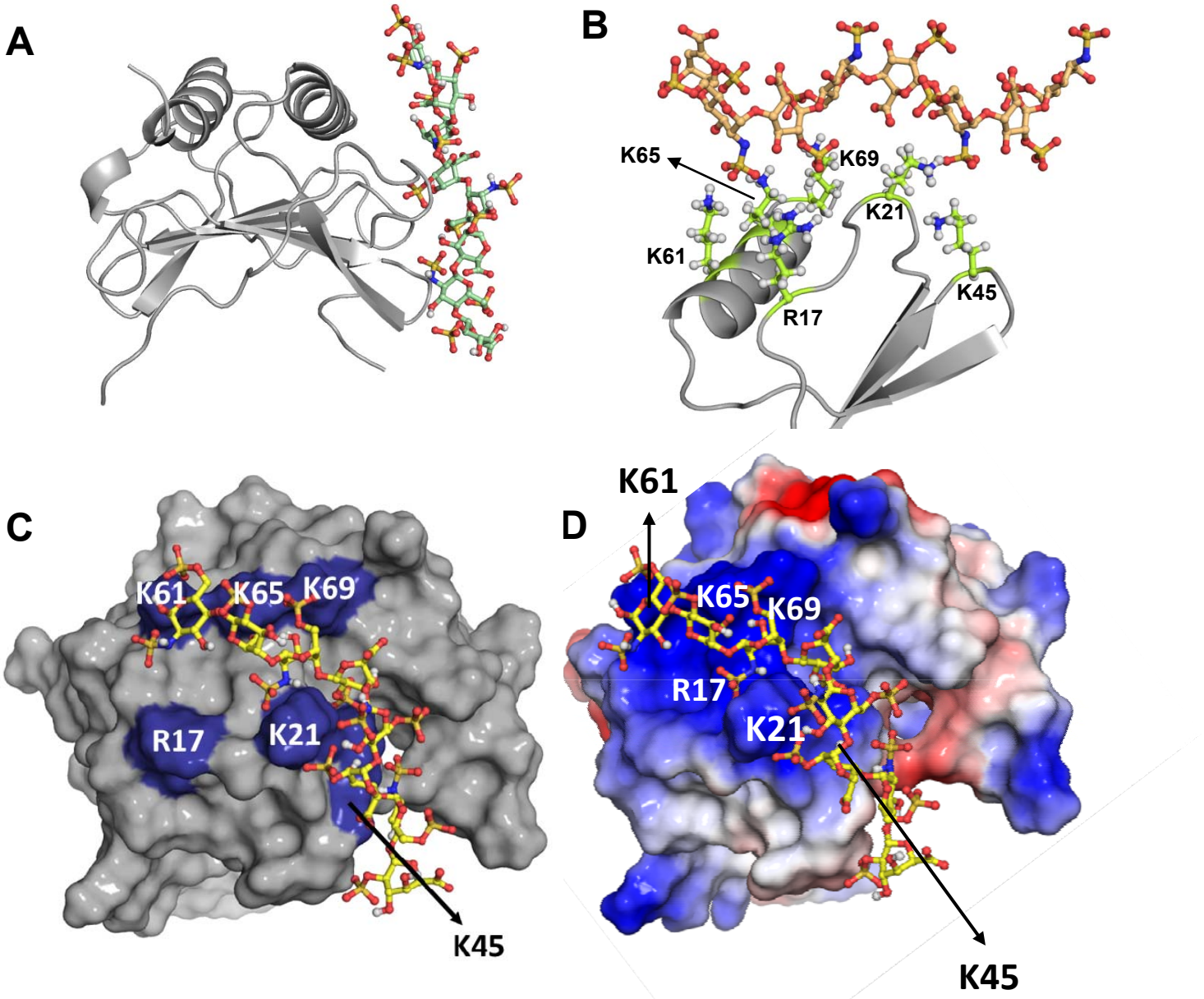
Titration	K_D (μM)	n	ΔH	-TΔS	ΔG
WT KC	3.8	2.1	-11.3	3.92	-7.40
WT MIP2	24.8	1.8	-4.32	-1.97	-6.28
KC-Dimer	4.21	2.0	-12.6	5.28	-7.33
MIP2-Dimer	3.88	2.0	-5.46	-1.93	-7.38

	B1	S1	B2	B3	B4	B5	B6	B7	B8	S2
MIP-2	R8	R17	x	K21	K45	x	x	K61	K65	K69
KC	R9		H20	K22	K46	R49	x	K61	K65	

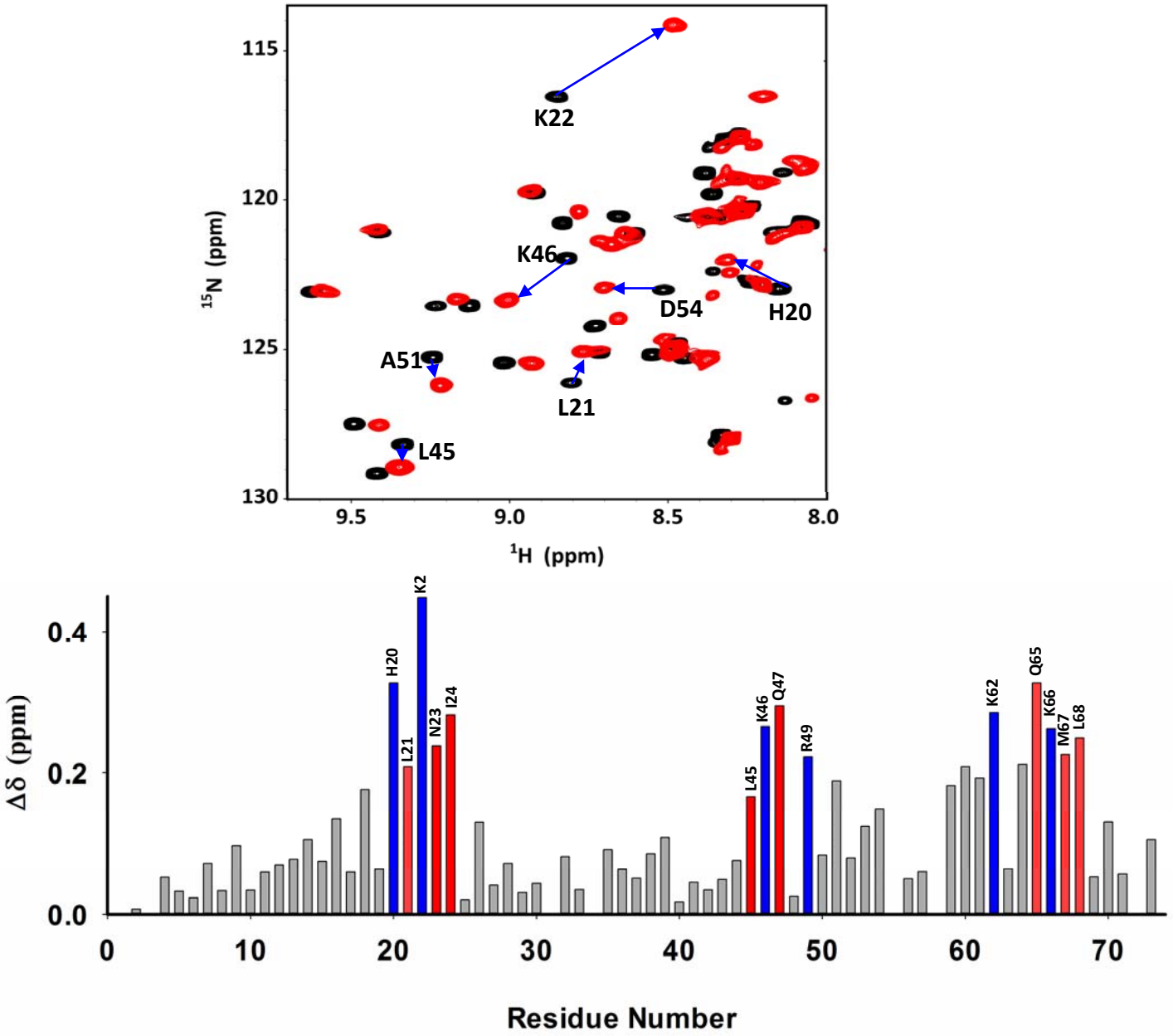
Structural basis of MIP2 binding to heparin dp8



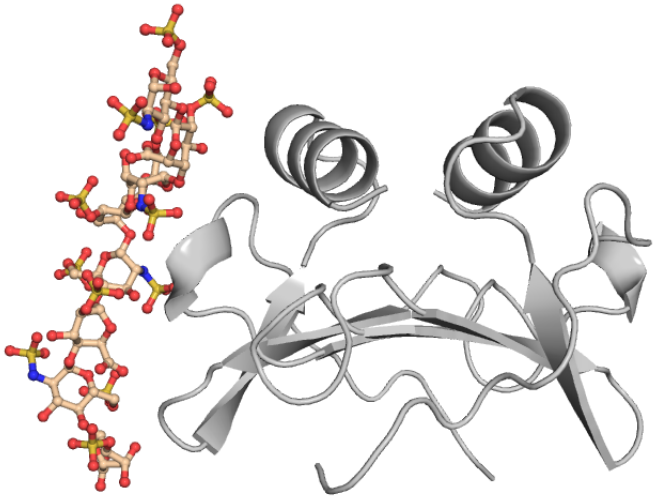
Structural Model of MIP2 binding to heparin dp8

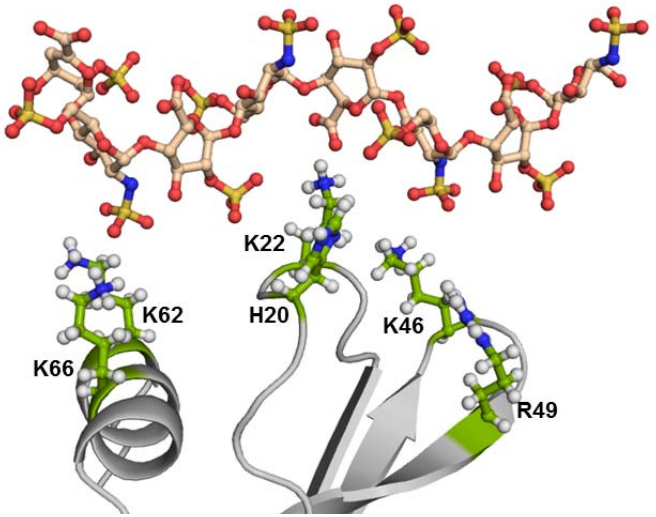


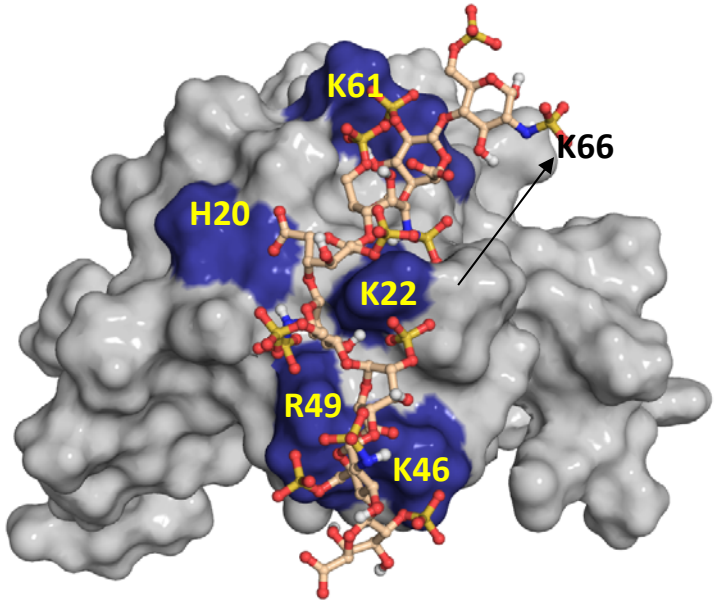
Structural basis of KC binding to heparin dp8

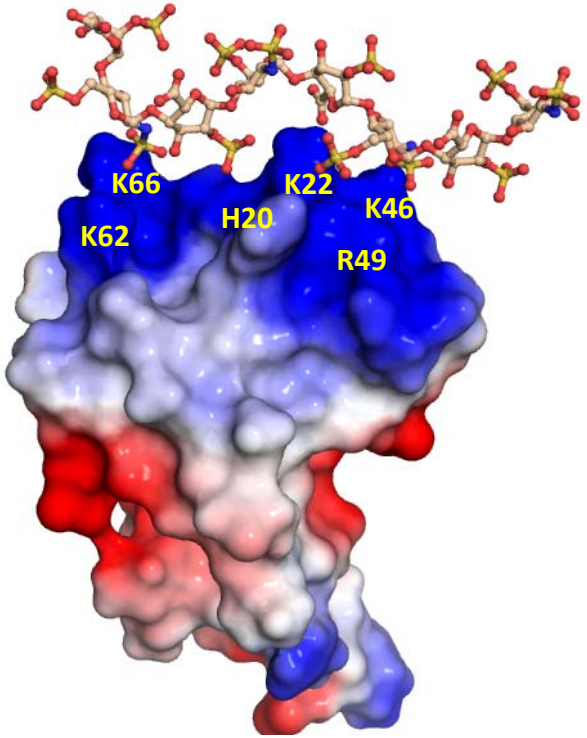


Structural Model of KC binding to heparin dp8



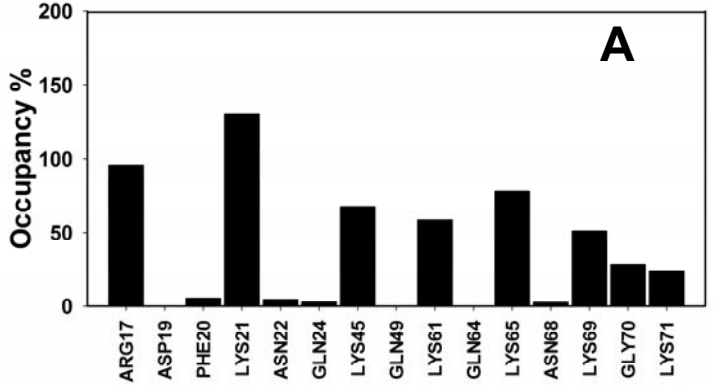


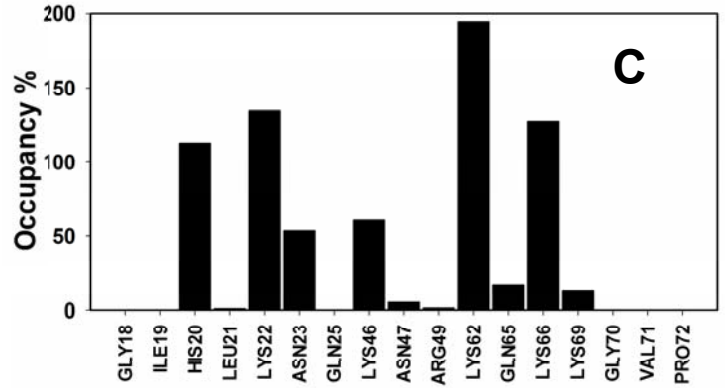




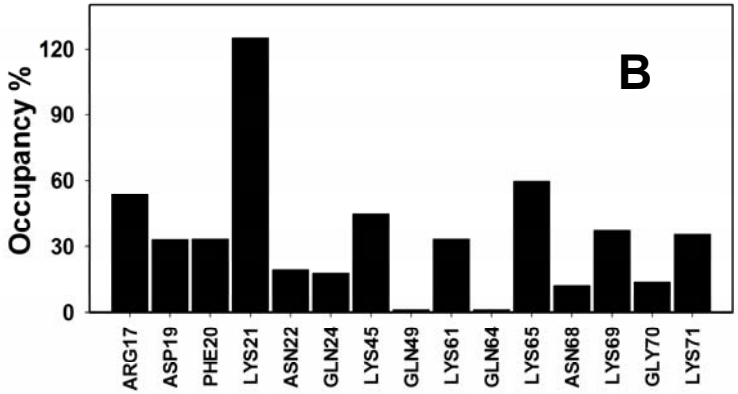
H-bonding properties from MD simulations

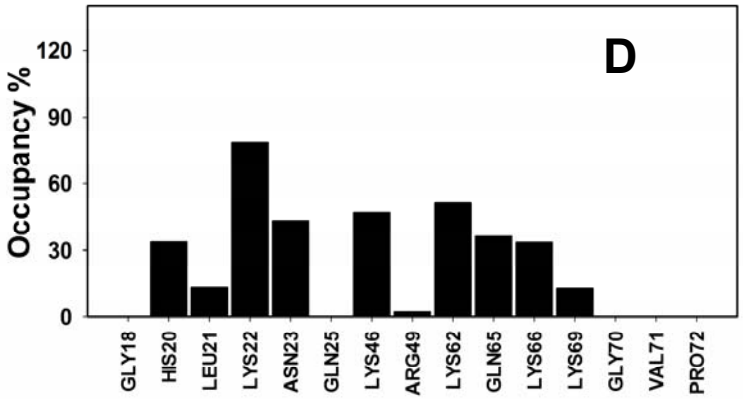
MIP2





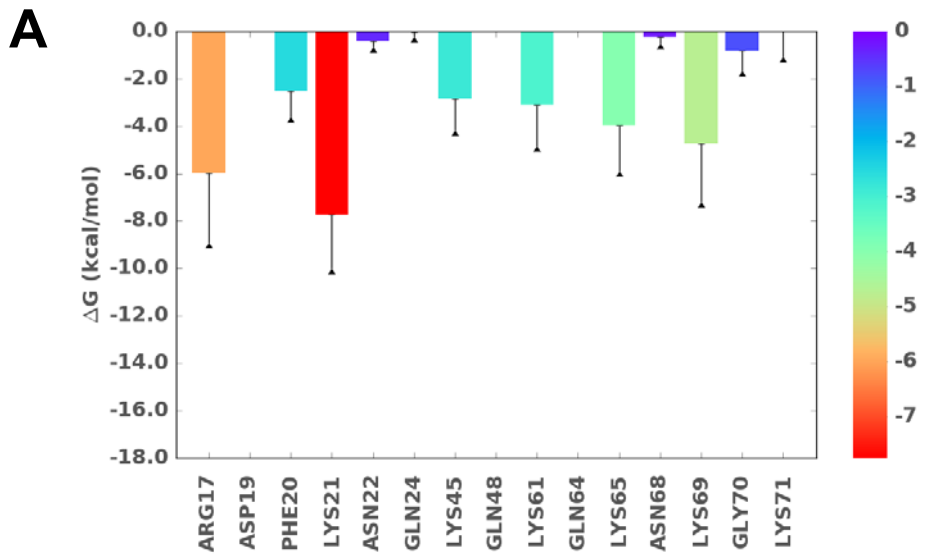
KC



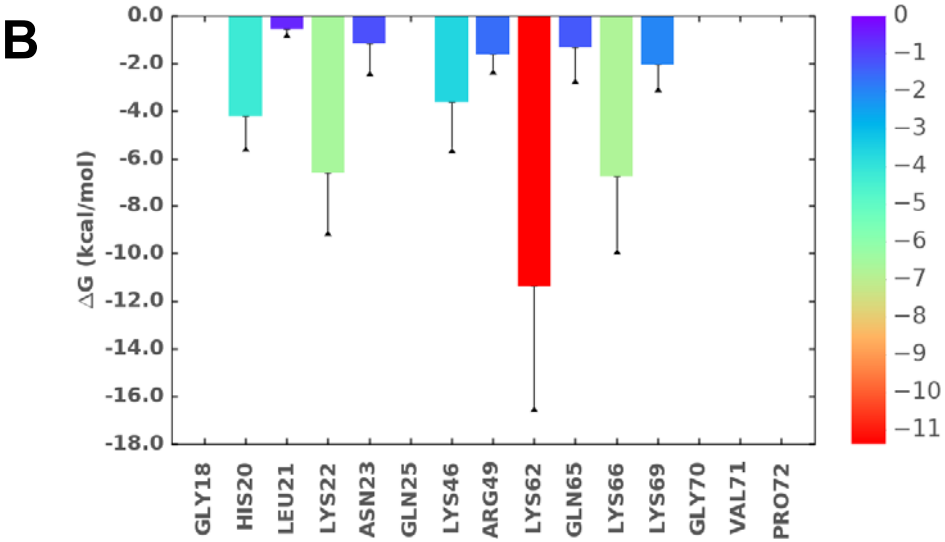


Binding energetics from MD simulations

MIP2



KC



ITC studies of CXCL8 to polymeric heparin (dp50)

IL8	Stoichiometry	L_c/n	Enthalpy (kcal/mol)	Entropy (cal mol^{-1} K^{-1})	Free energy (kcal/mol)
WT	37±4	1.36±0.14	-92.7±1.7	-81.1±1.0	-11.6±0.2
R26C Dimer	49±2	1.01±0.04	-92.1±1.7	-77.8±1.1	-14.3±0.1
V27E29P Monomer	22±1	2.29±0.15	-73.9±2.6	-64.5±1.2	-9.4±0.1

The data indicate that the interactions between IL8 and dp_{50} heparin is driven by enthalpy.

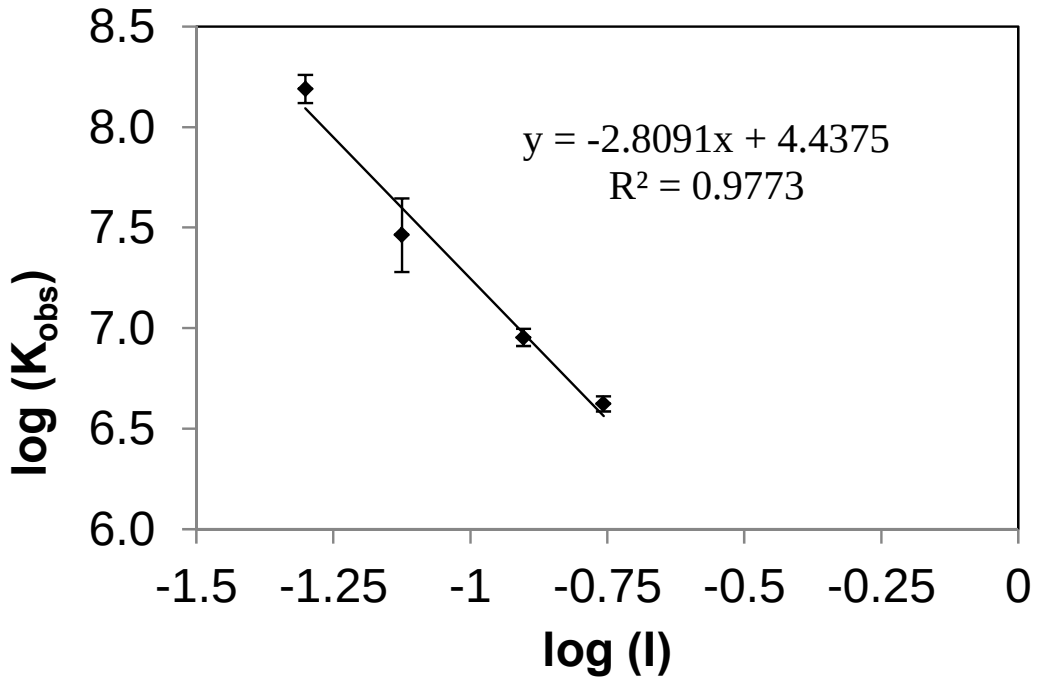
L_c/n indicates the number of saccharide units required to bind with a single IL-8 monomer.

The data indicate that ~ two saccharide units bind with an R26C dimer or V27E29P monomer.

Energetically, the interactions between dimer and WT are almost similar.

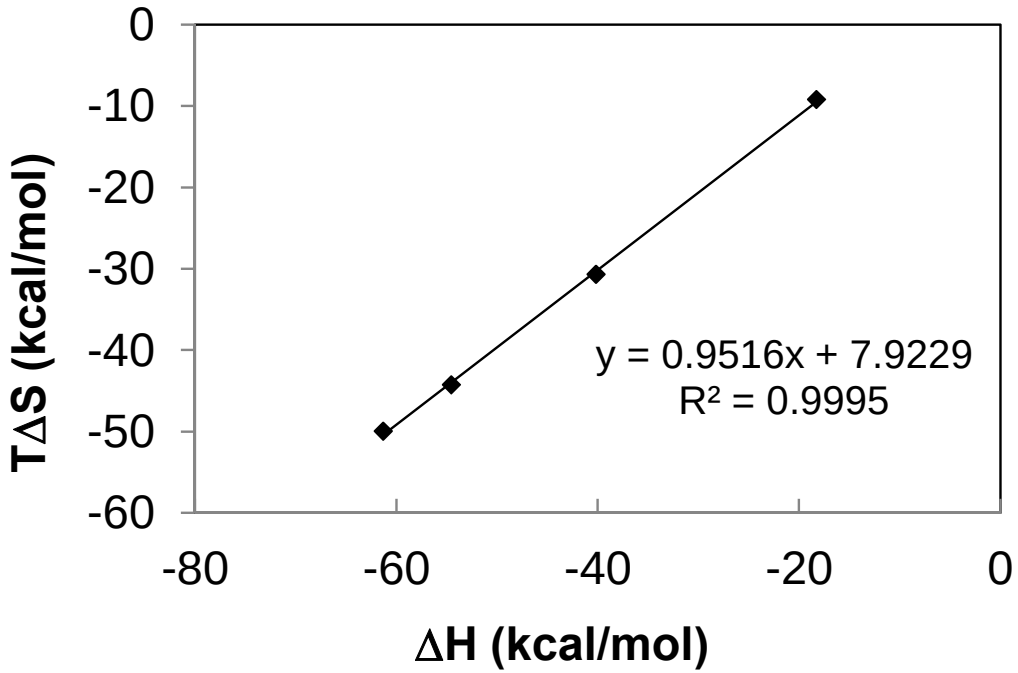
Experimental condition: 50 mM HEPES, pH 7.5, 25º C

Contributions from Non-ionic interactions



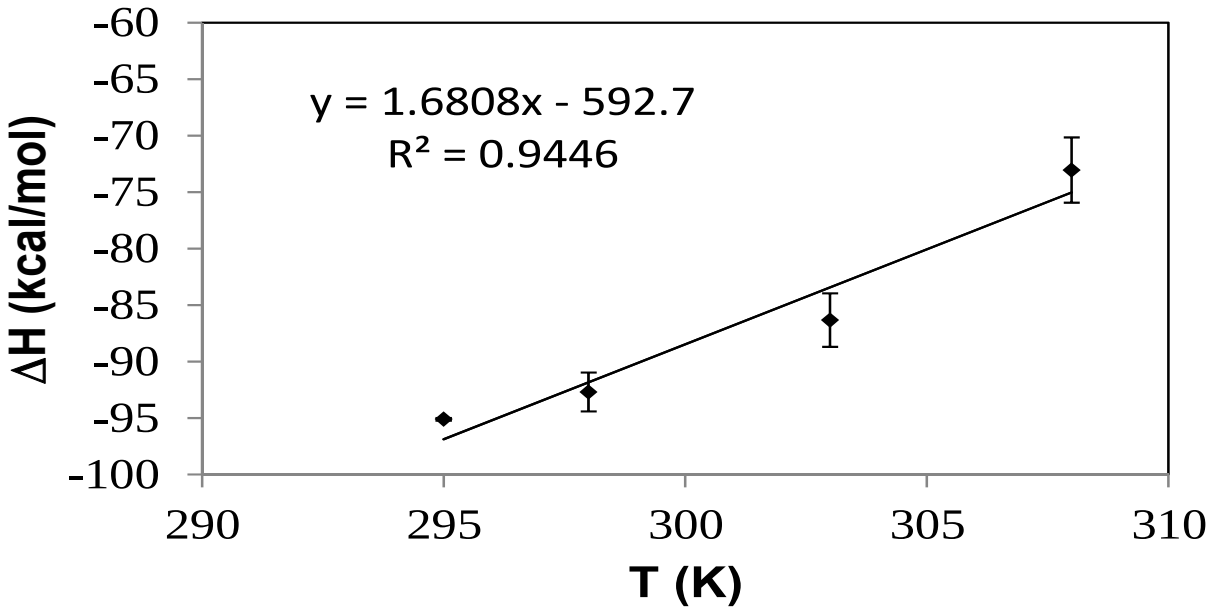
Interactions between WT IL8 and dp50 heparin were studied at different ionic strength (I) to get the non-electrostatic contribution towards total free energy using the following equation: $\log (K_{a,obs}) = \log (K_{a,nel}) - N \log (I)$. The free energy from non-electrostatic interactions were -6.1 kcal/mol.

Enthalpy-entropy compensation



Due to increase in ionic strength, the interactions became weaker resulting in reduced enthalpic contribution.
The decrease in enthalpy were compensated by increase in enthalpy possibly due to release of counter ions.

Effect of Temperature



The interactions between IL-8 and dp_{50} were studied at different temperature (T). The slope of the plot of ΔH vs. T indicate the change in heat capacity (ΔC_P) for the system. The ΔC_P obtained was 1.7 kcal mol^{-1} K^{-1}. The positive ΔC_P indicates burial of polar residues.

Thermodynamics of CXCL8-heparin interactions

- Interactions are predominantly enthalpically driven
- Thermodynamic contribution (enthalpy and entropy) vary between related chemokines
- The stoichiometry is two heparins per dimer for most chemokines studied to date.
- The thermodynamic parameters are influenced by experimental conditions which could be the reason for wide variation in reported Kd values in literature.
- Binding is mediated via both ionic and non-ionic interactions.
- The change in heat capacity of the system is positive indicating burial of polar residues.