

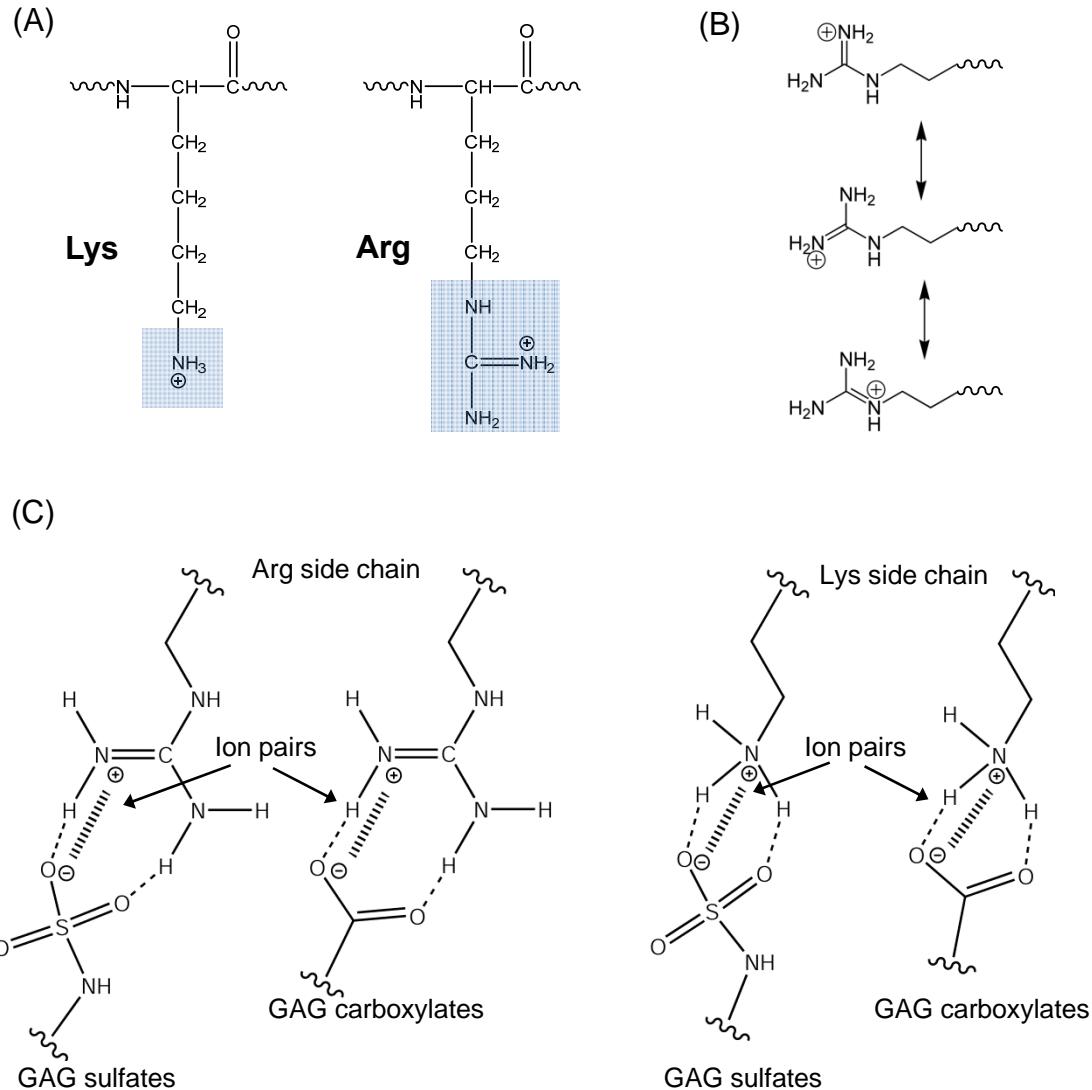
**MEDC 691 Special Topics – Glycochemistry and Glycobiology
Richmond, VA**

Thermodynamics of protein-GAG complexes

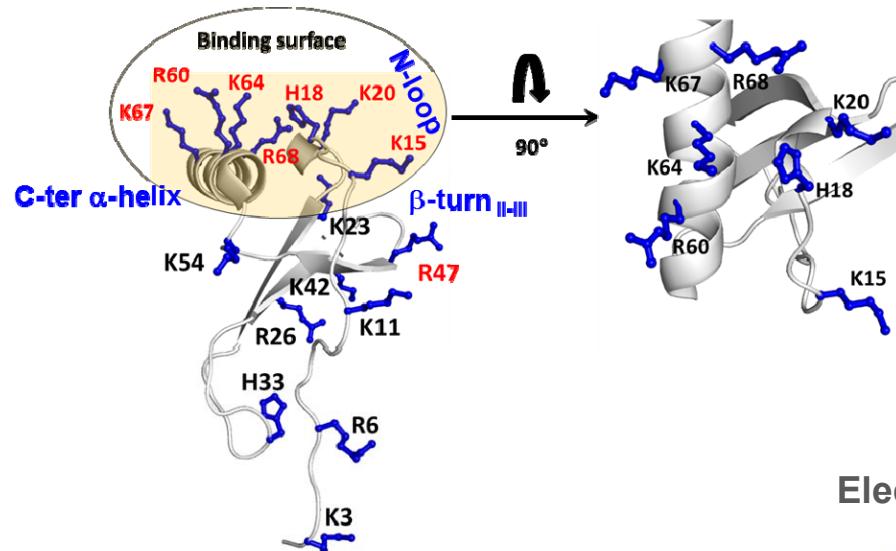
**Krishna Rajarathnam, UTMB
8th April 2019**



Role of electrostatics: residue-specific interactions

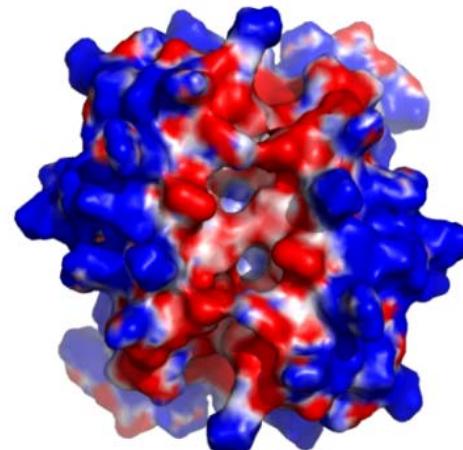


GAG Binding Surface of Interleukin-8/CXCL8

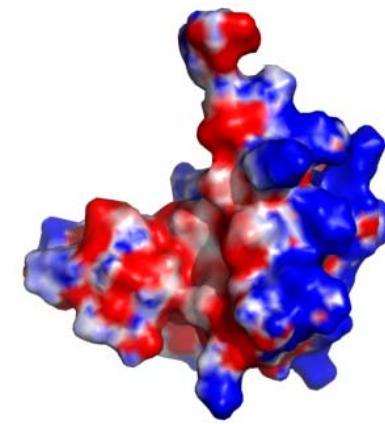


Electrostatics Architecture of Interleukin-8

Unique vs. Multiple binding sites

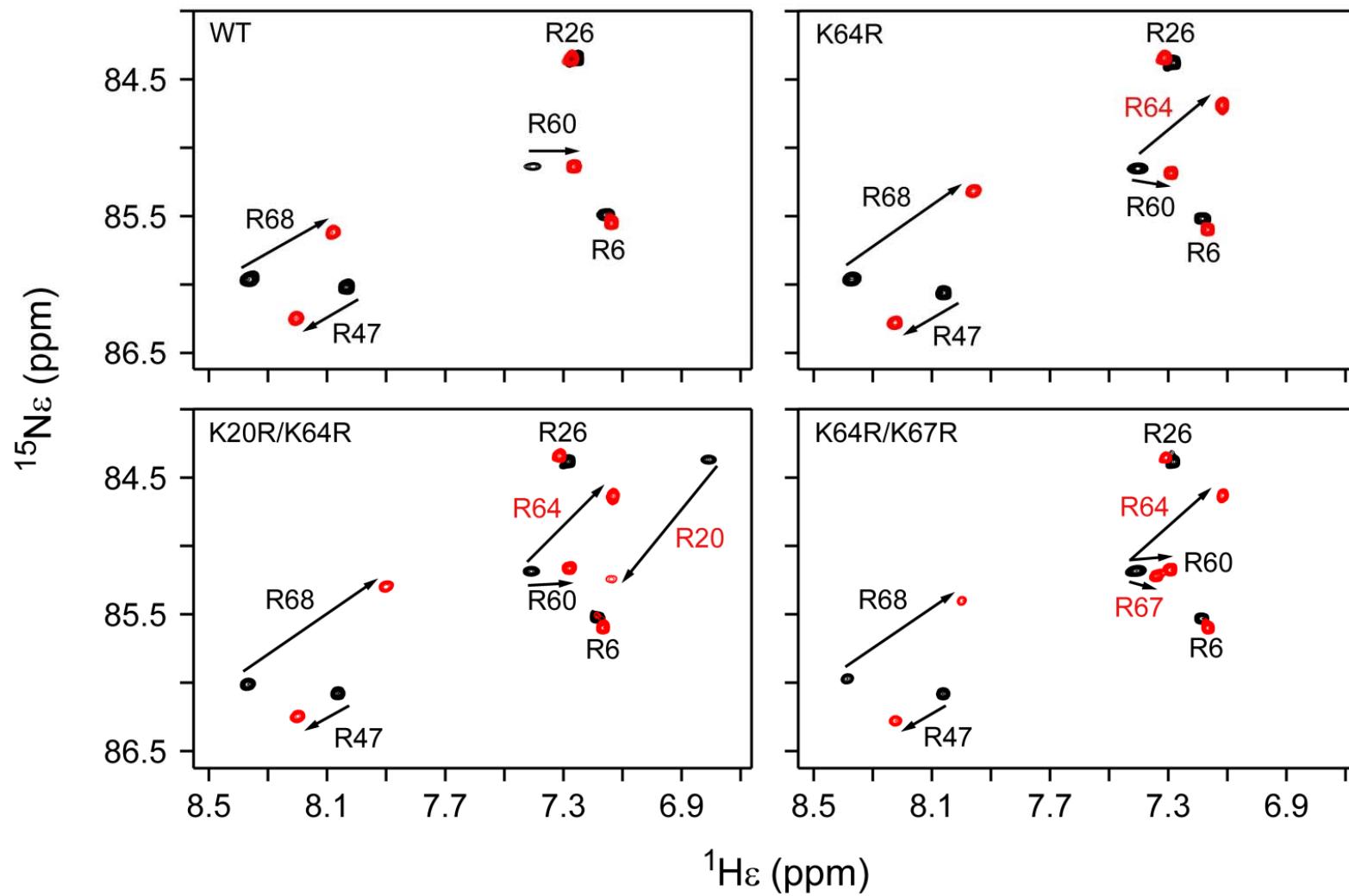
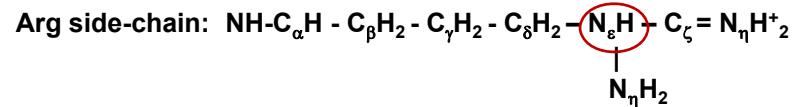


Dimer

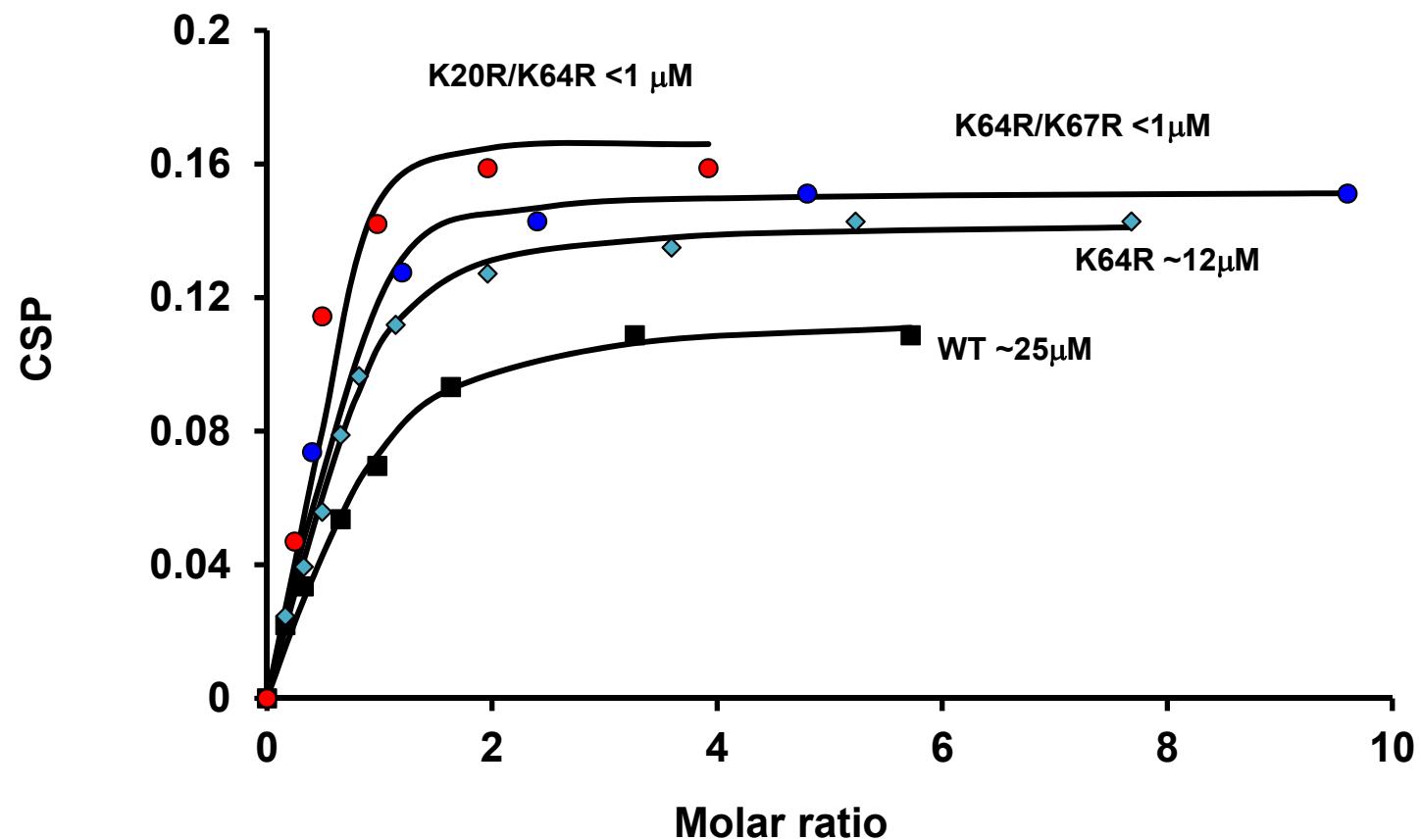


Monomer

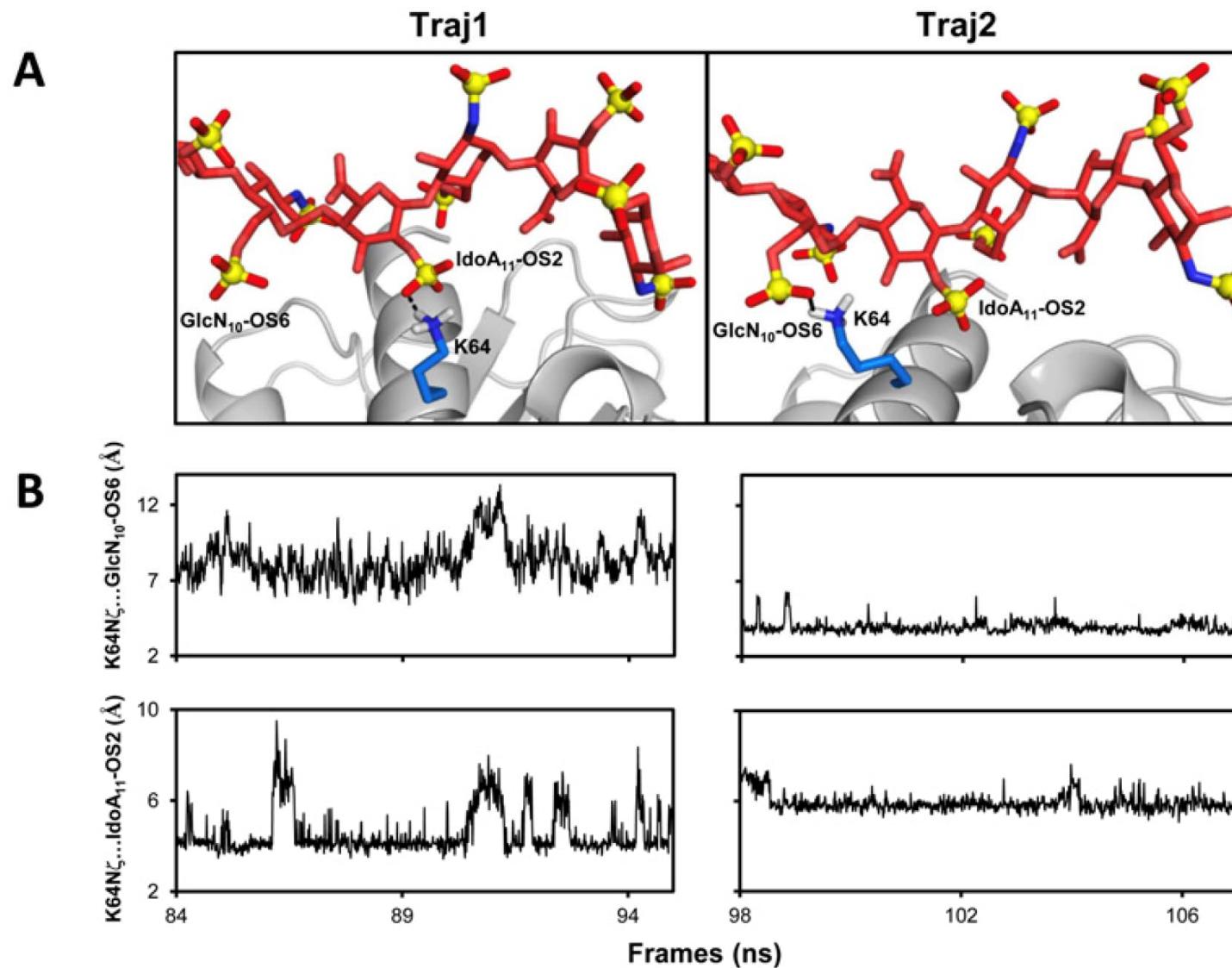
Role of electrostatics – Arginines



Arginines vs. Lysines: Affinity

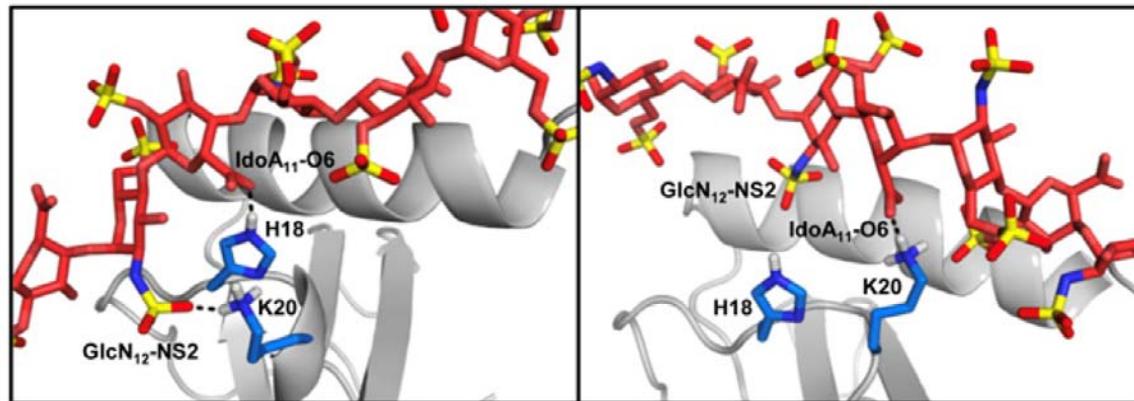


MD data showing a single residue interacting with different GAG side chain groups

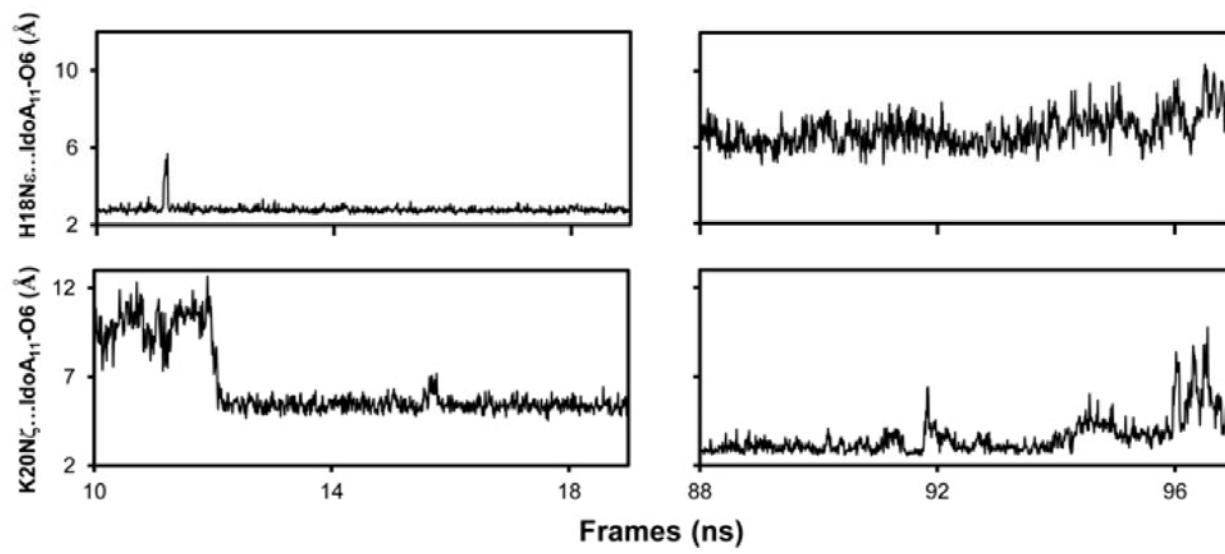


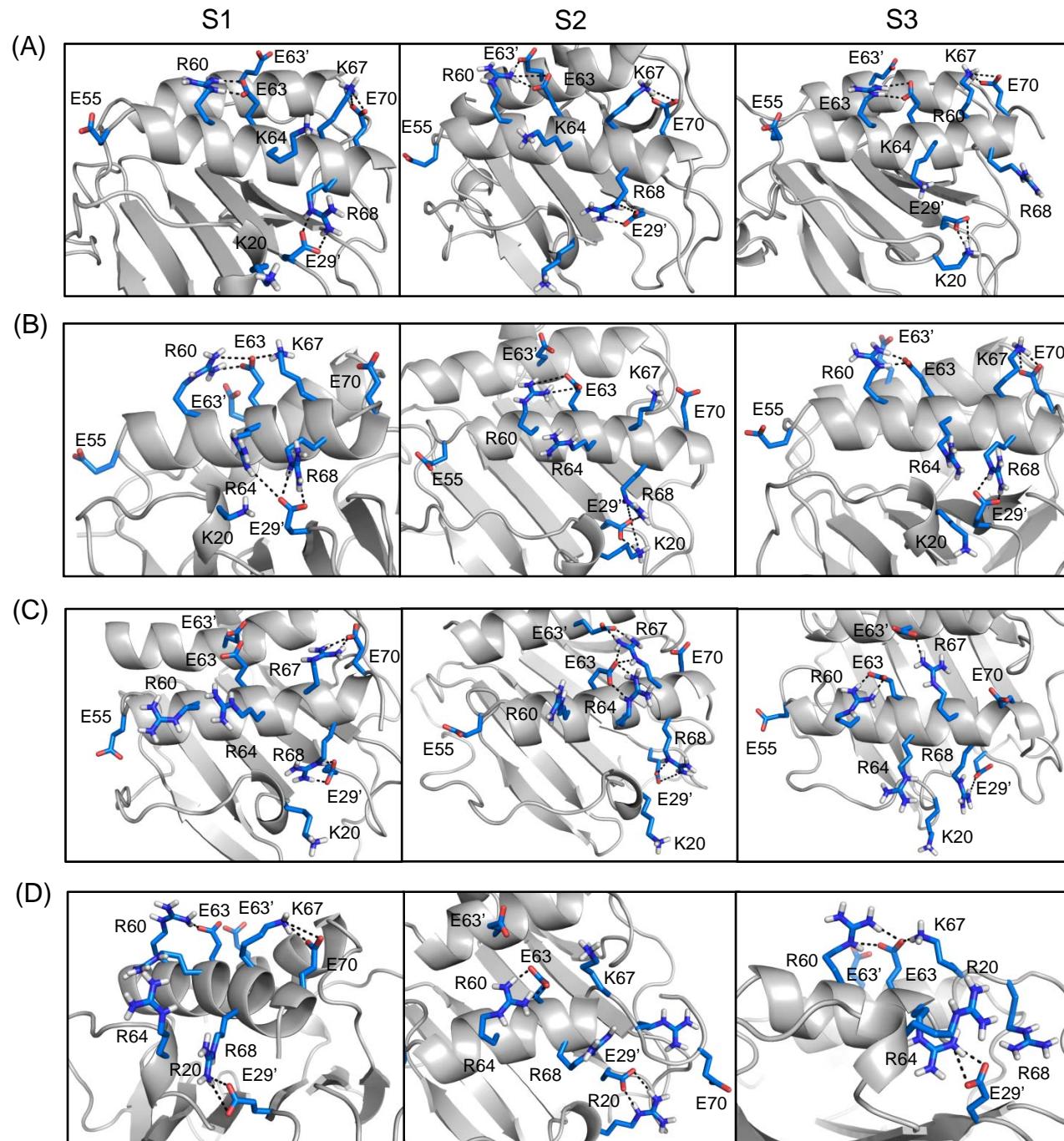
MD data showing a single GAG side chain group interacting with different protein side chain residues

C

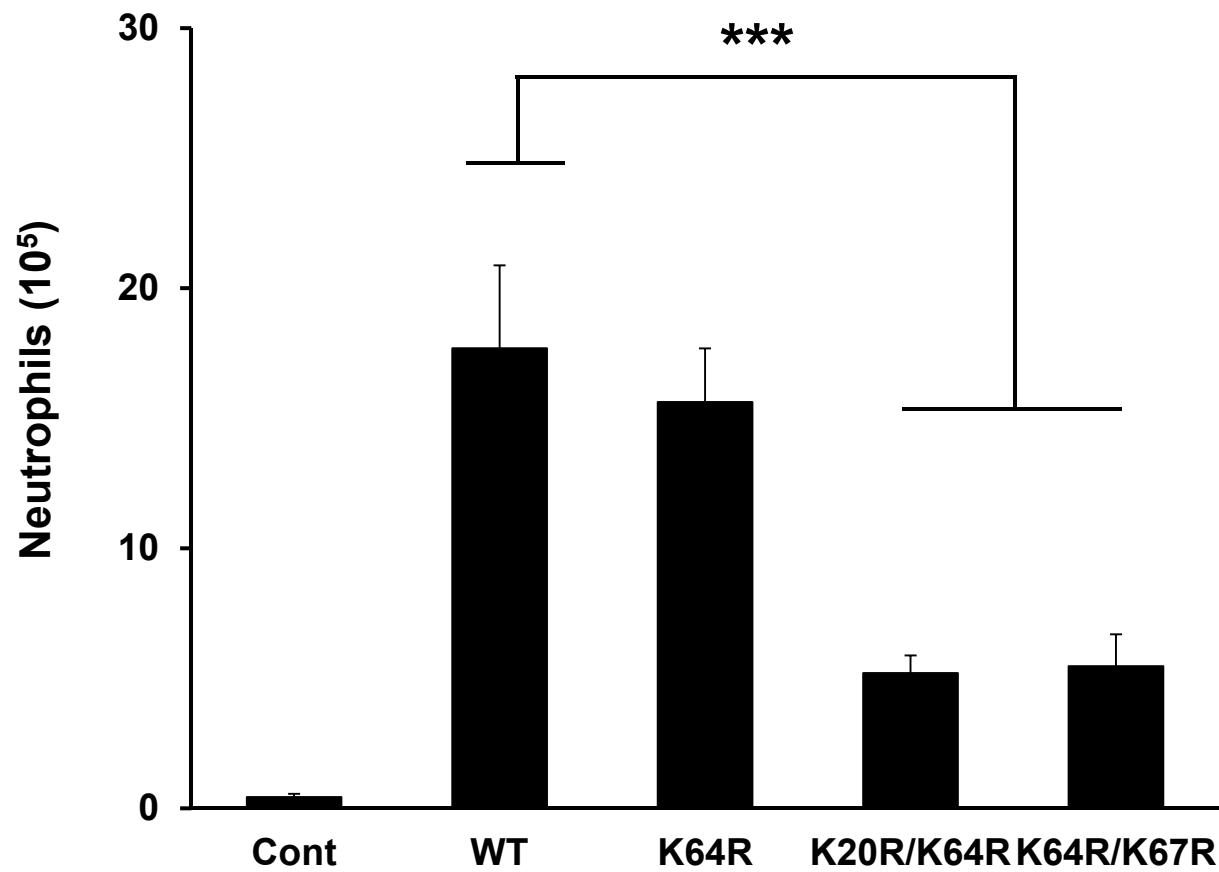


D





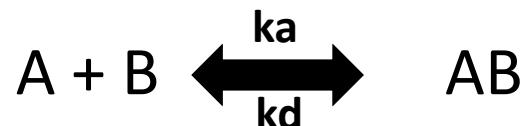
GAG Affinities: Function



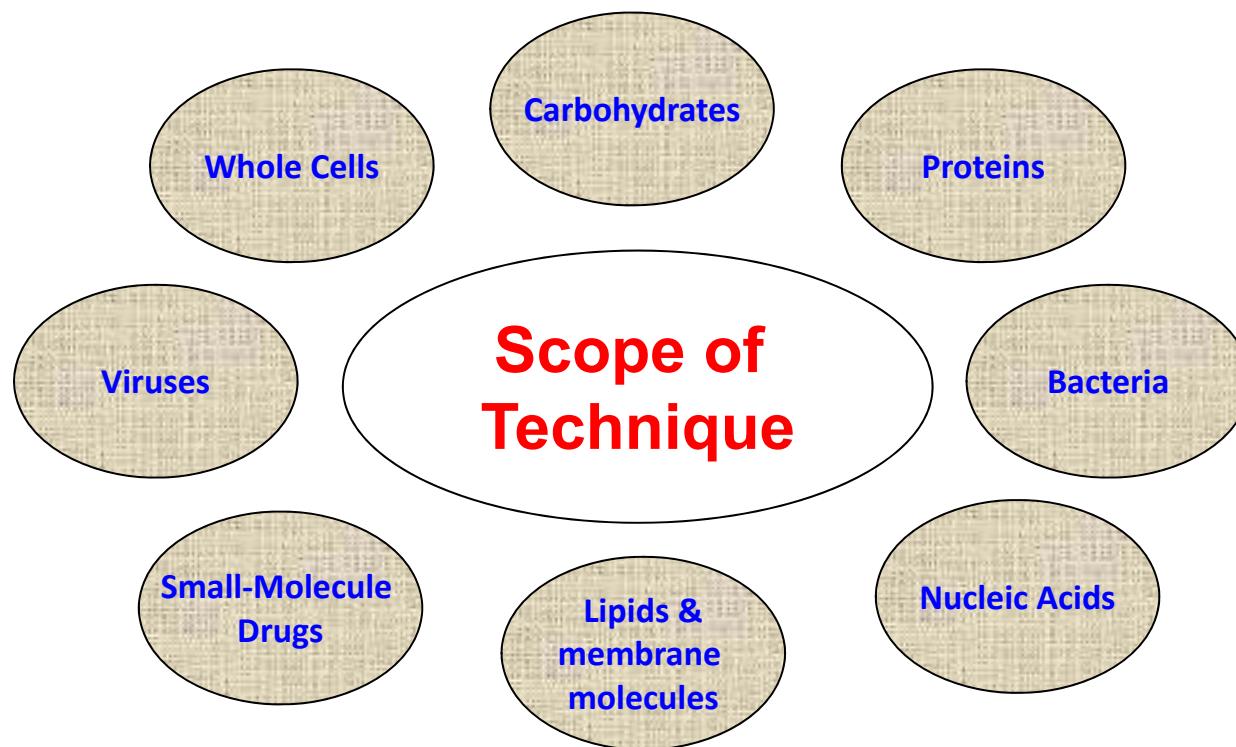
Kinetics -- SPR for the study for biological interaction analysis

- Does the interaction happen?
- How fast? How slow?
- How strong?
- How much?
- What is interaction?

Specificity	Concentration,
Kinetics	Immunogenicity
Affinity	SPR-MS



What can be studied?



Biomolecular Interaction Analysis: BIAcore

- Detection principle: Surface Plasmon Resonance: SPR
- One binding partner (LIGAND) immobilised on chip
- Other (ANALYTE) injected: microfluidics
- PC collects binding data in *real time*
- Chip is regenerated to remove analyte
- Cycle is repeated

Comprehensive information

- Molecular interactions in real time:

Detect

Yes/No

Identify

Specificity

Binding partners

Characterize

Affinity

Kinetics

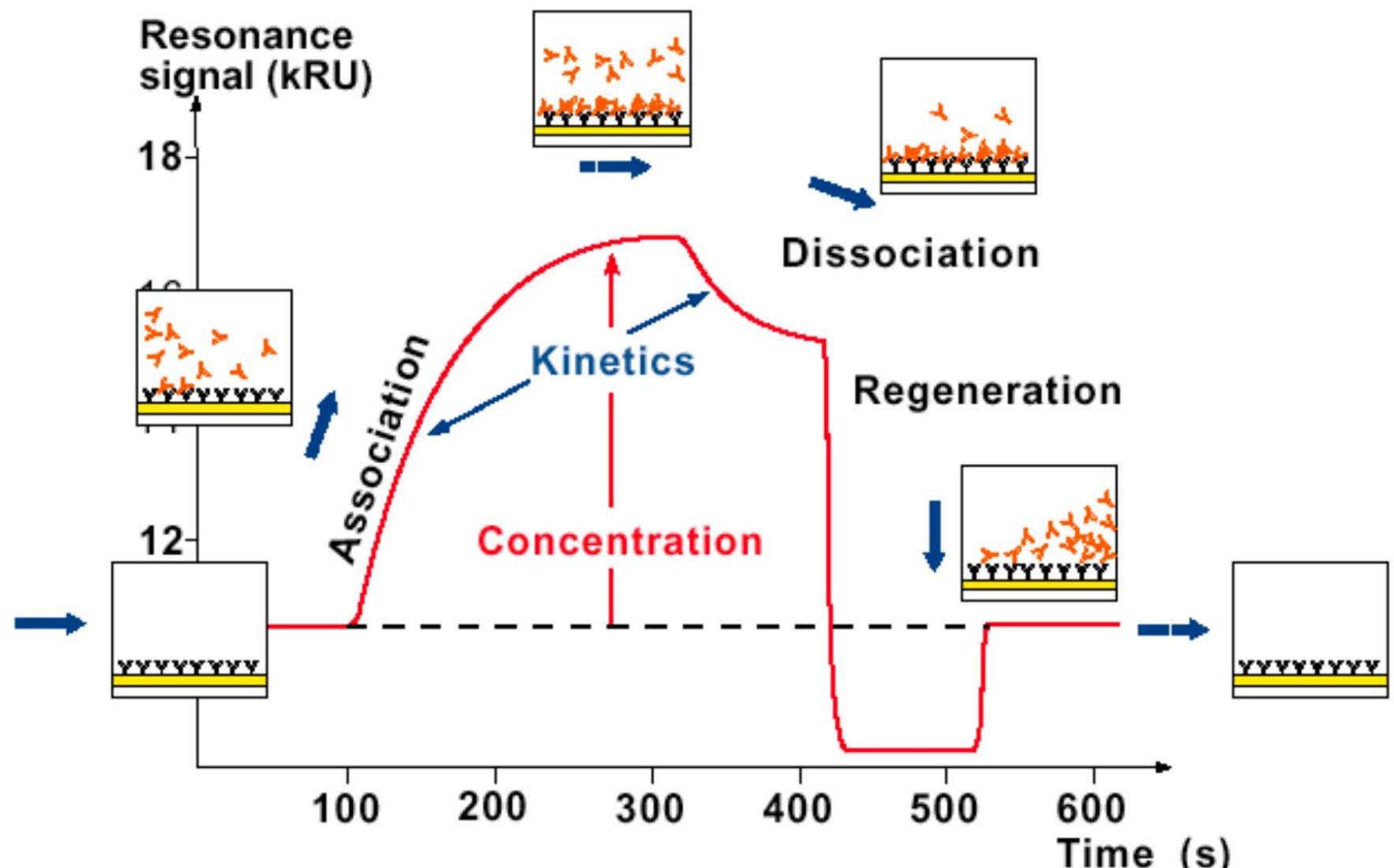
Epitope mapping

Concentration

Thermodynamics

Mass Spec Link-Up

The Sensogram



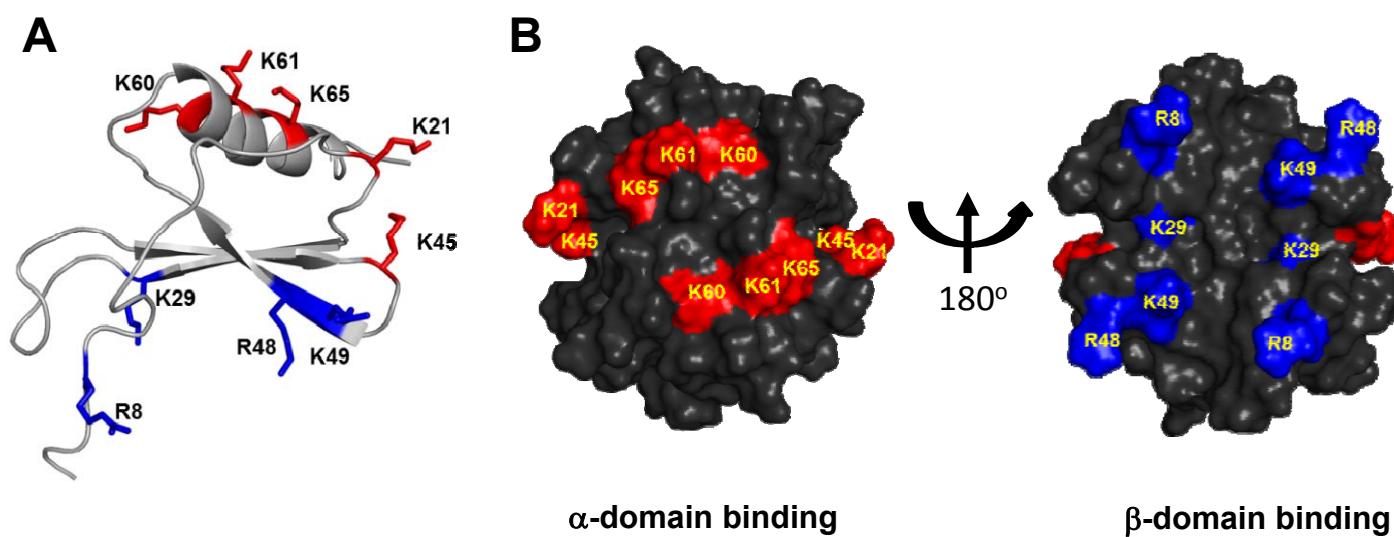
Advantage of SPR

- Ability to perform real-time measurement:
 - Insight to dynamic nature of binding system and layer formation
- Use of selective slides to study binding events:
 - Eliminate the need for labeled reactants
- Exceptional sensitivity:
 - Small quantities of purified reagents are required

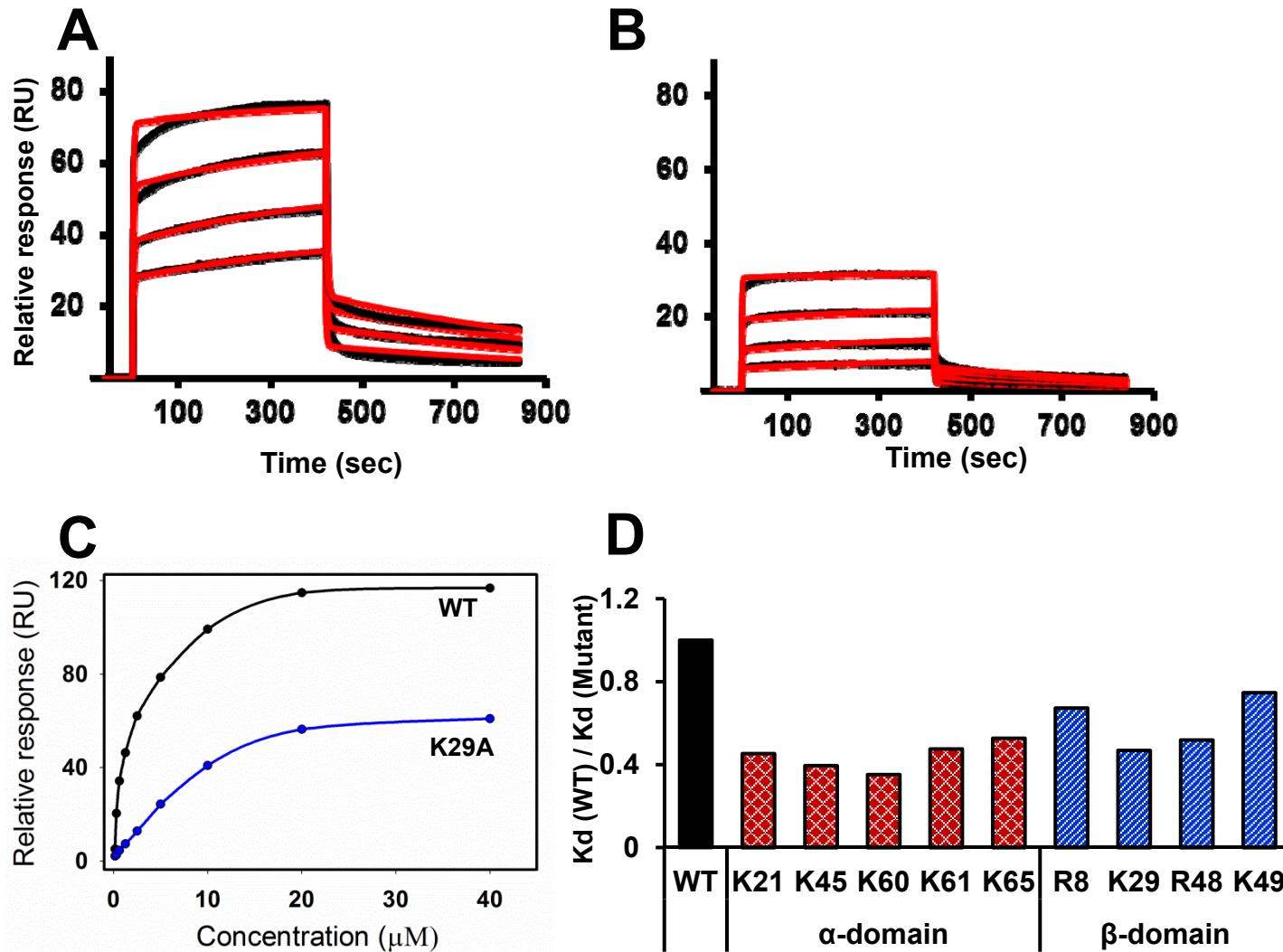
Limitations

- Mass transport limitations
- Can take time to optimise regeneration conditions
- Sorting problems can be time consuming

Heparin binding to CXCL1: Two non-overlapping domains span the dimer interface



Kinetic evidence that residues from both domains are involved in heparin binding



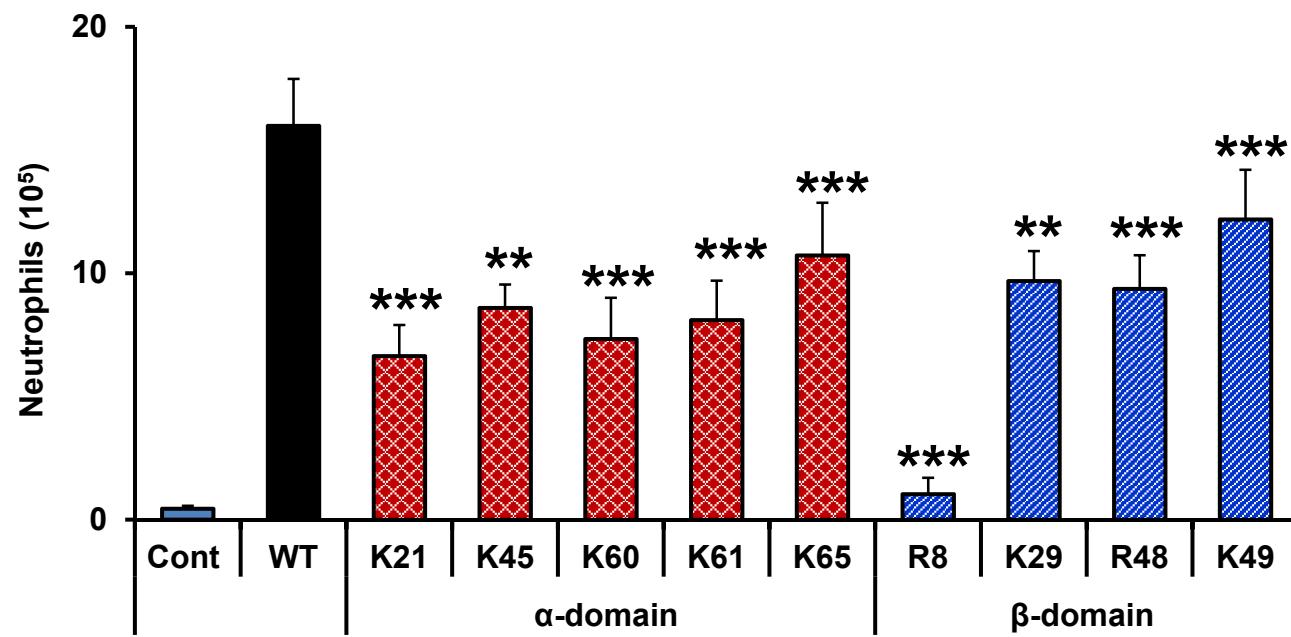
SPR analysis of CXCL1 and mutants with heparin^a

Protein	$k_{on} \cdot (M^{-1} \cdot s^{-1})$	$k_{off} \cdot (s^{-1})$	$k_{off}/k_{on} = K_d \cdot (μM)$	χ^2
WT	2.73 X 10⁴	0.0271	1.0	8.4
K21A	1.61 X 10 ⁴	0.1477	9.1	4.1
R8A	2.81 X 10 ⁴	0.0868	3.1	5.8
K29A	2.02 X 10 ⁴	0.0947	4.7	5.8
K45A	2.51 X 10 ⁴	0.1360	5.4	4.8
R48A	1.02 X 10 ⁴	0.0547	5.6	8.8
K49A	2.08 X 10 ⁴	0.1680	8.4	2.8
K60A	1.26 X 10 ⁴	0.0822	1.8	2.9
K61A	3.31 X 10 ⁴	0.2291	6.5	3.4
K65A	1.26 X 10 ⁴	0.0746	5.9	2.7

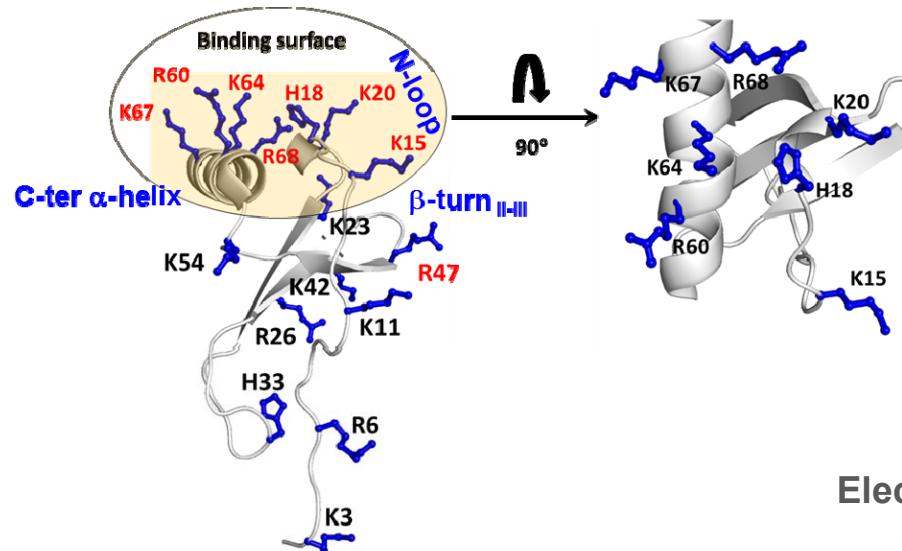
J

^aBinding affinities were calculated from rate of association (k_{on}) and the rate of dissociation (k_{off}) where where $K_d = k_{off}/k_{on}$ and by steady state analysis. χ^2 values were provided as a measurement of the quality of the fit between experimentally derived data and data from binding models.

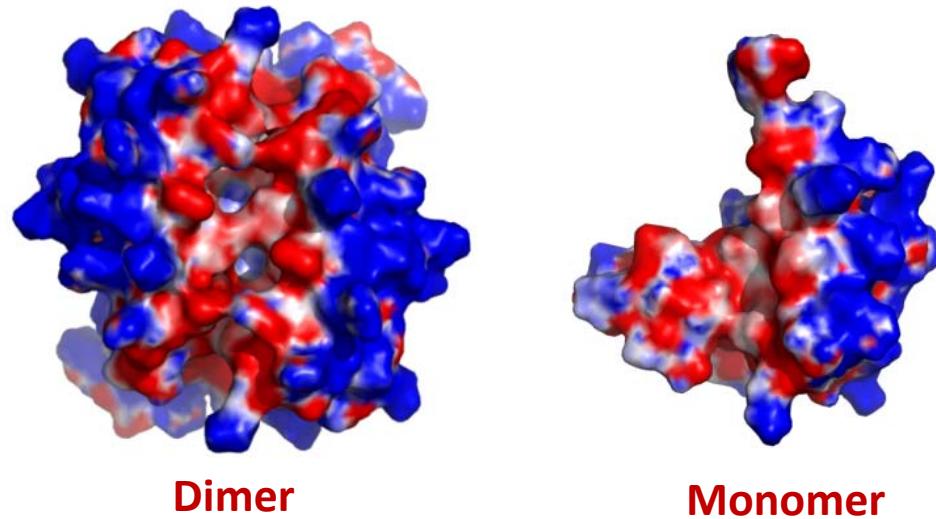
CXCL1 GAG mutants are less active in a mouse model



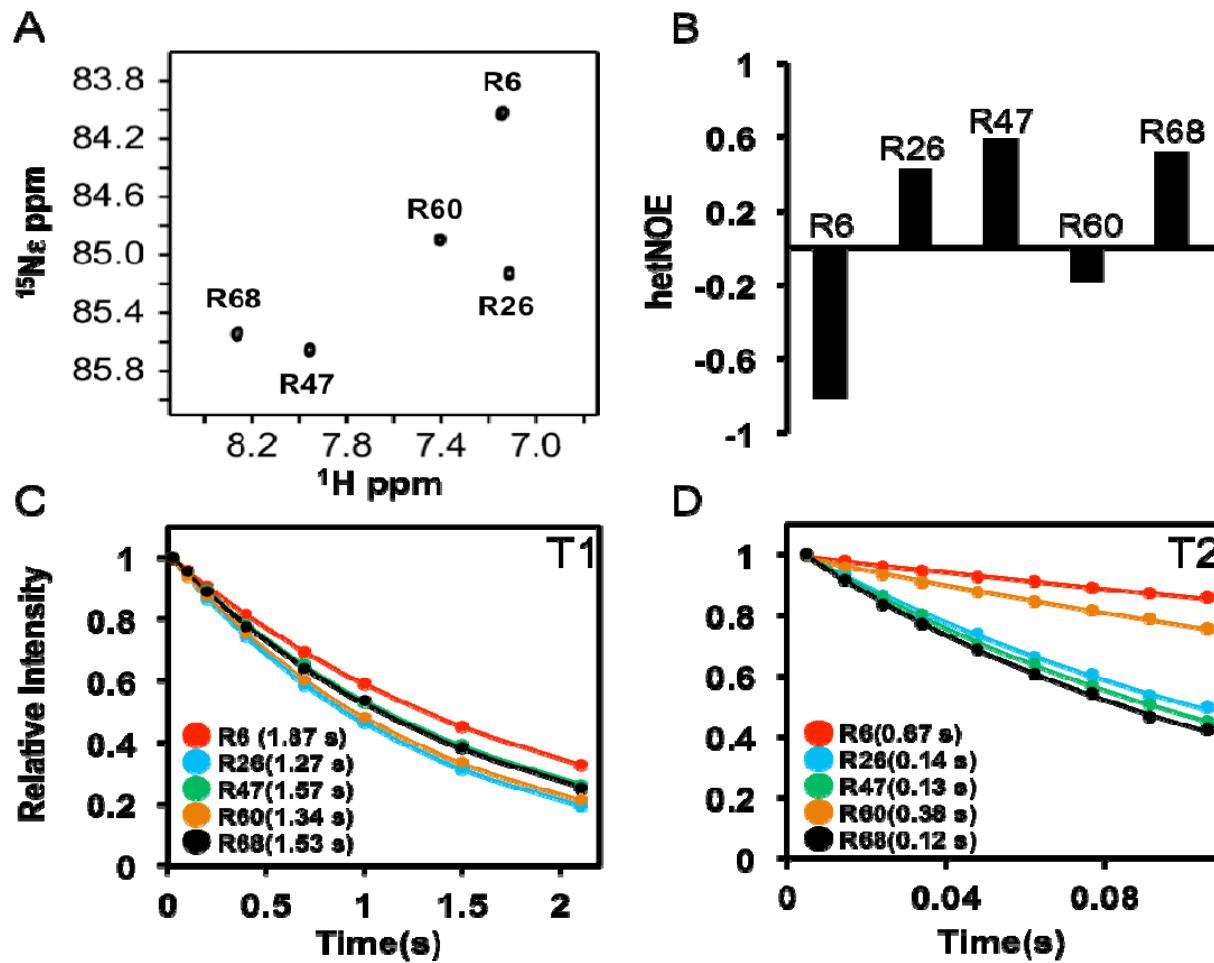
GAG Binding Surface of Interleukin-8/CXCL8



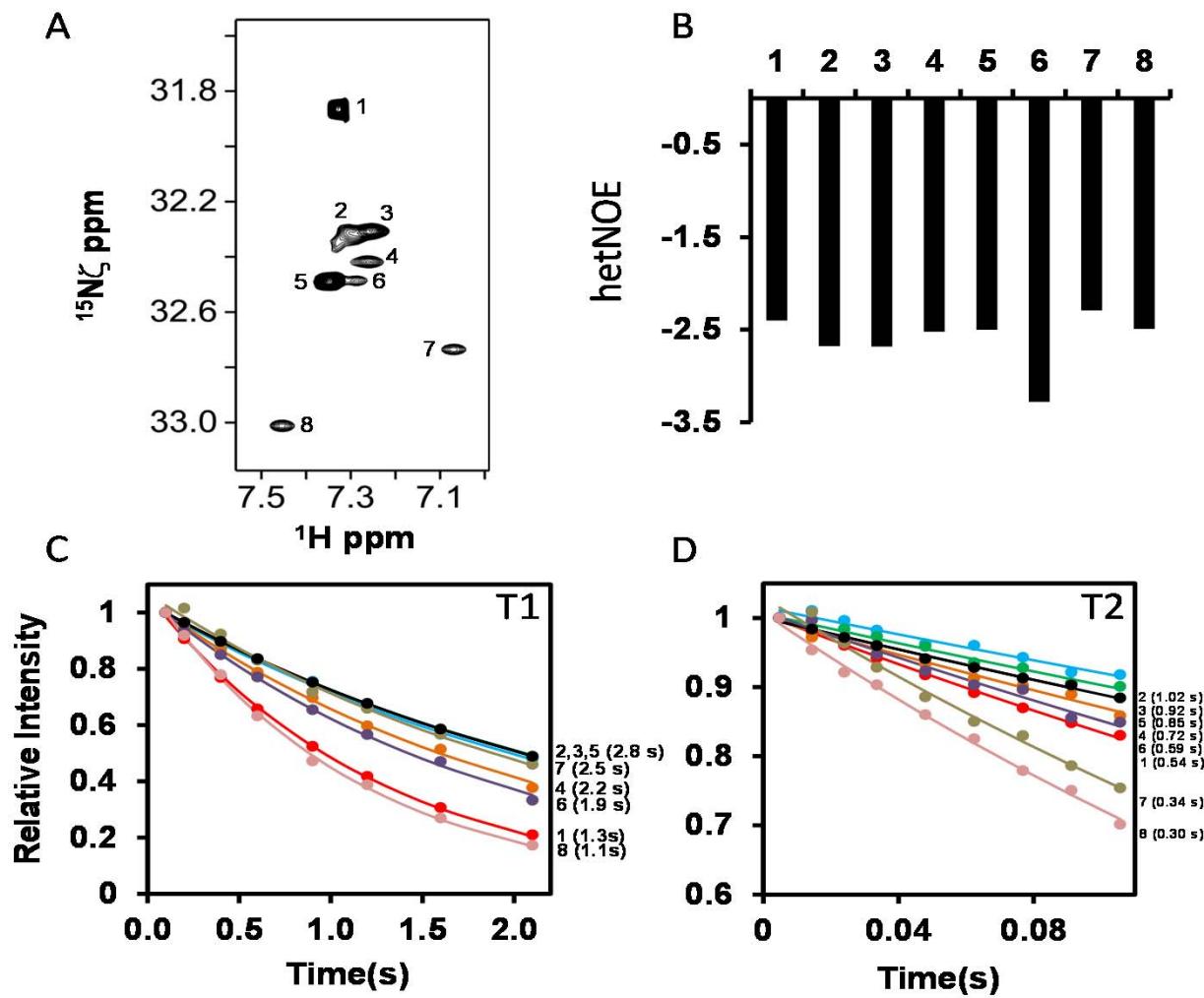
Electrostatics Architecture of Interleukin-8



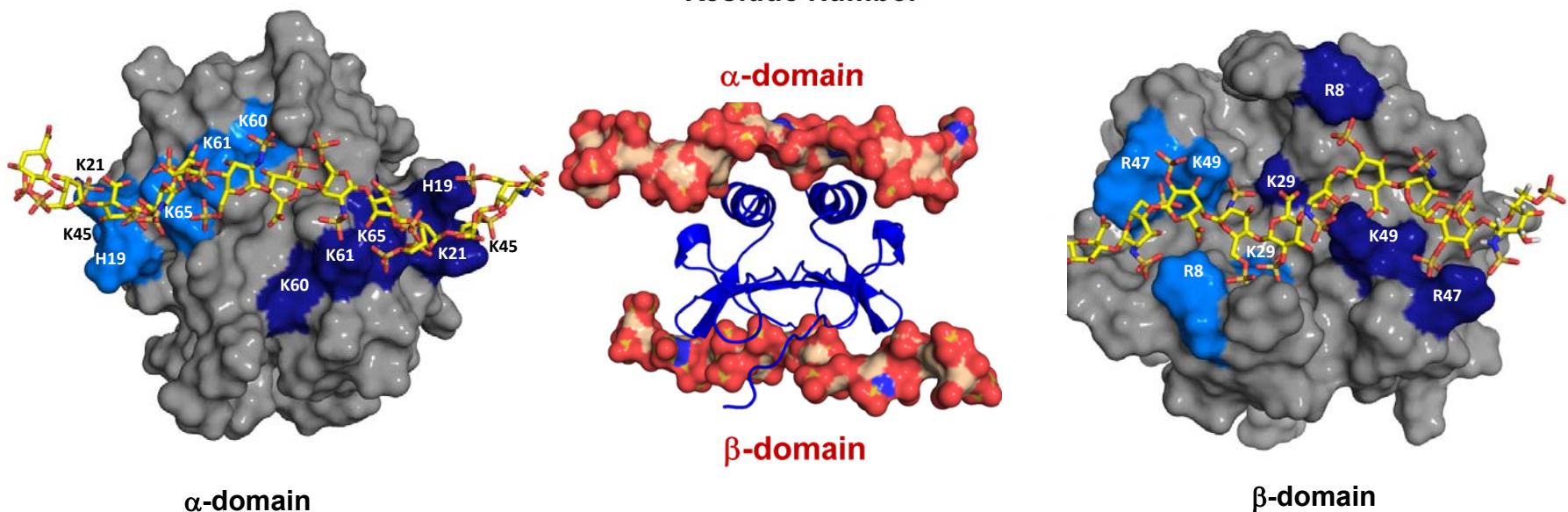
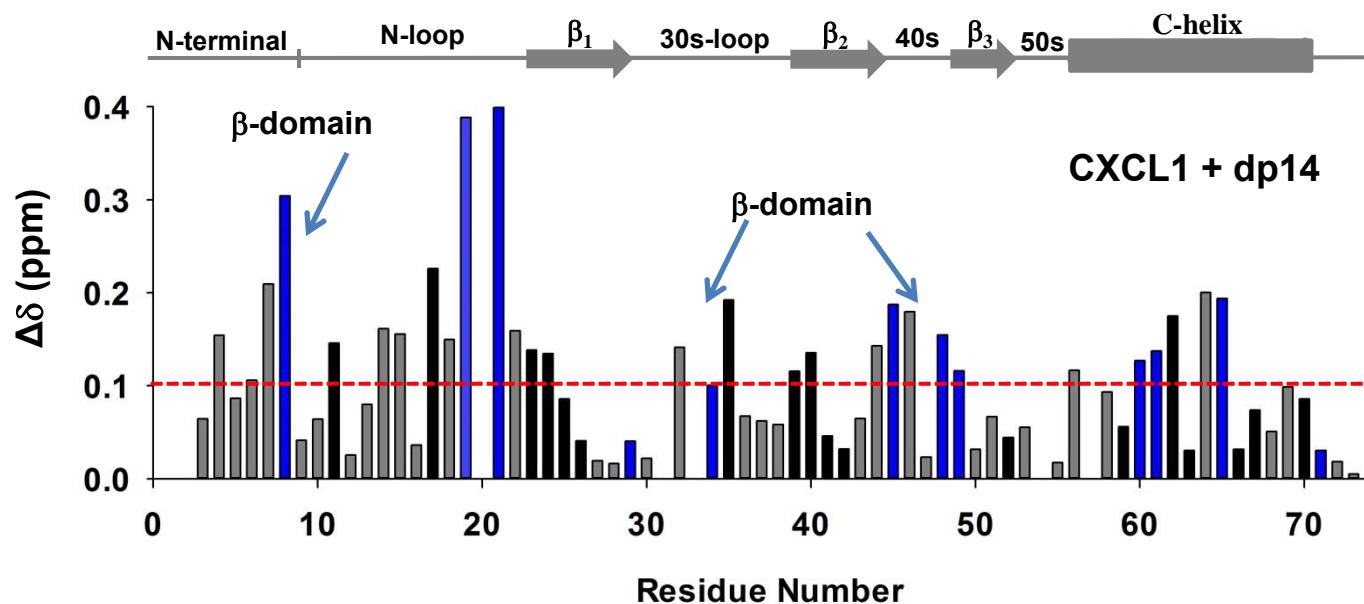
Dynamics of Lysine and Arginine Side Chains – NMR studies



Dynamics of Lysine and Arginine Side Chains – NMR studies

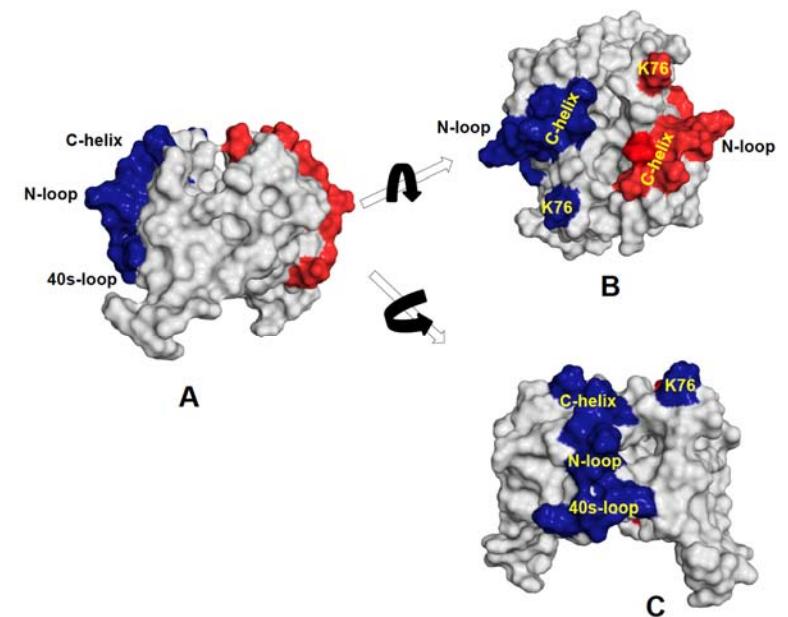
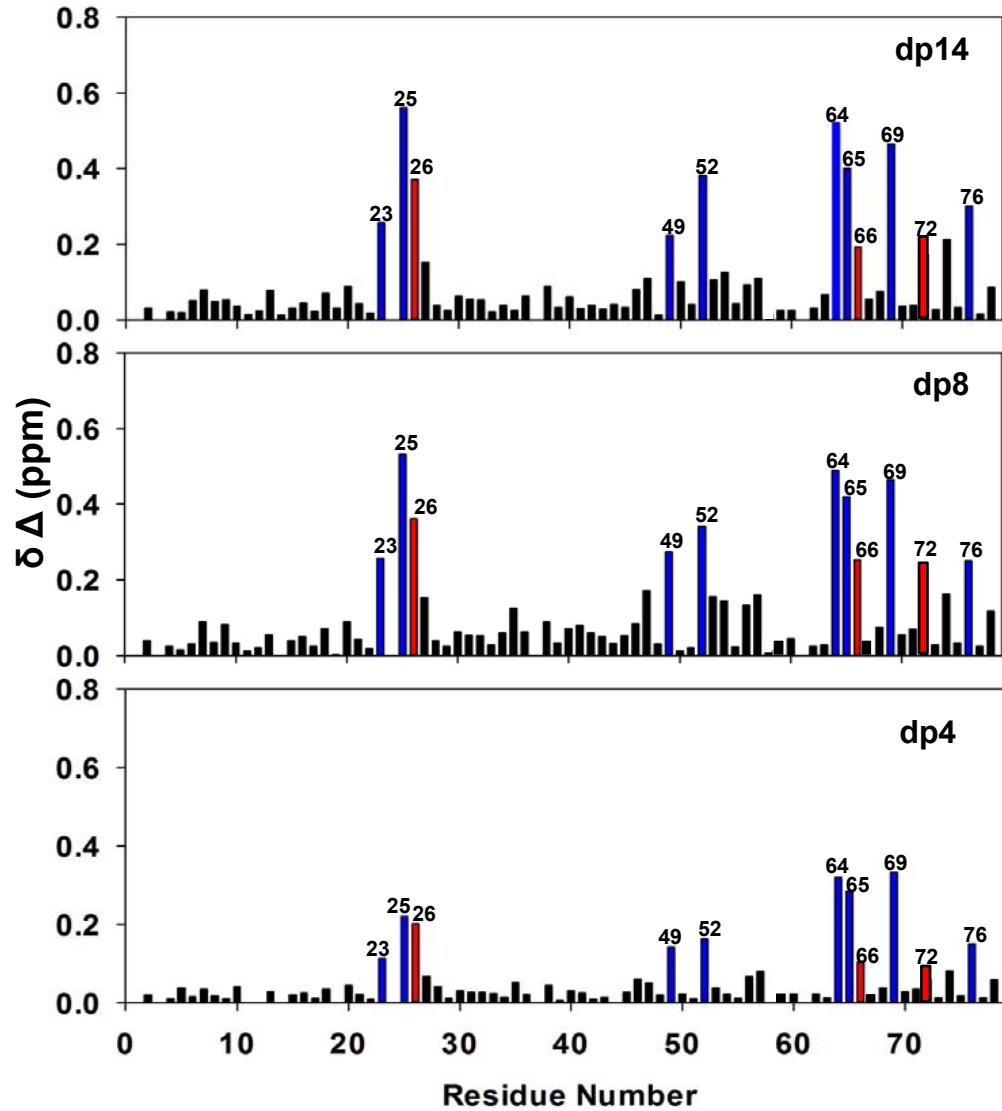


CXCL1 binds two distinct domains and spans the dimer interface



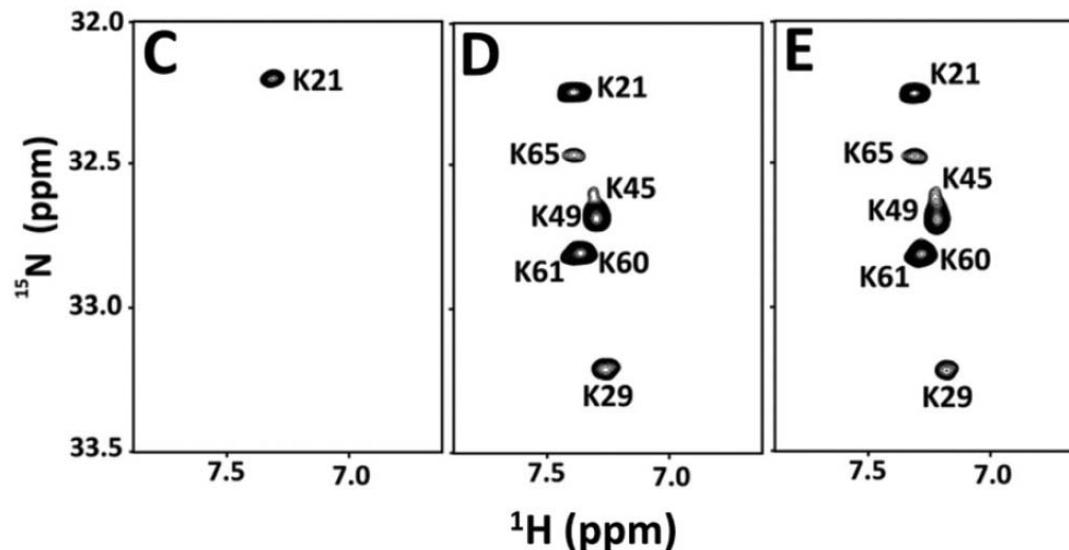
Sepuru & Rajarathnam, JBC, 2016

GAG-CXCL5 dimer Interactions: Binding does not span the dimer interface

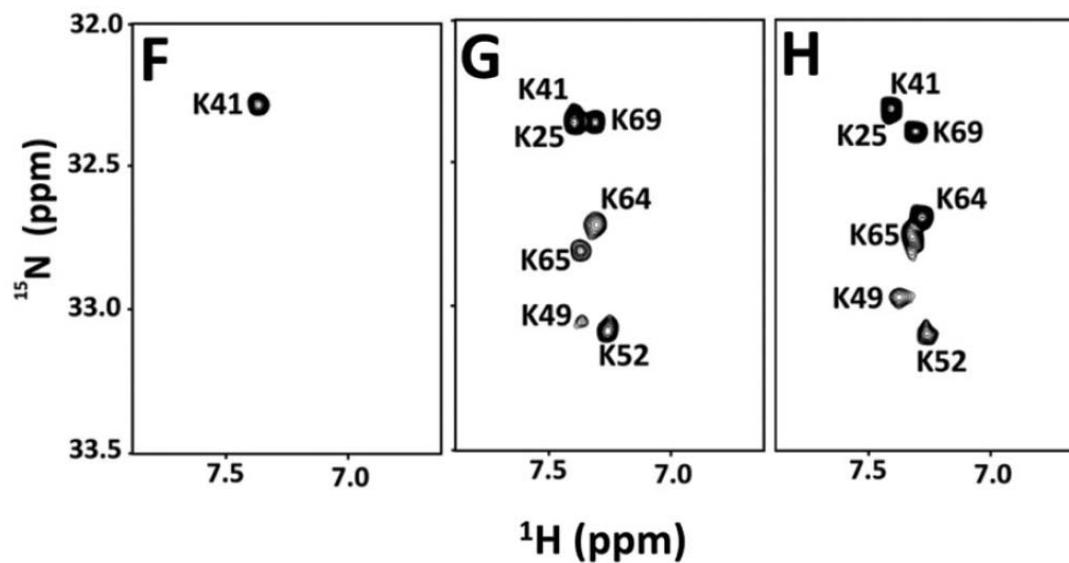


How does lysine dynamics influence GAG binding – NMR studies – HISQC spectra

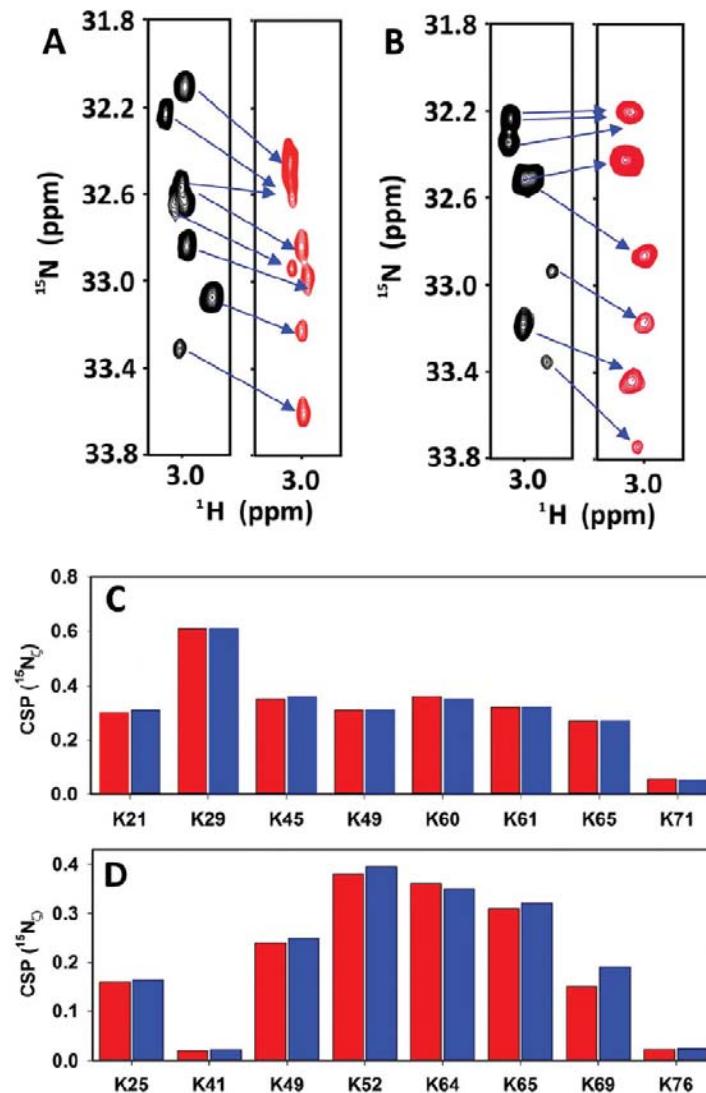
CXCL1



CXCL5



How does lysine dynamics influence GAG binding – NMR studies – H₂CN spectra



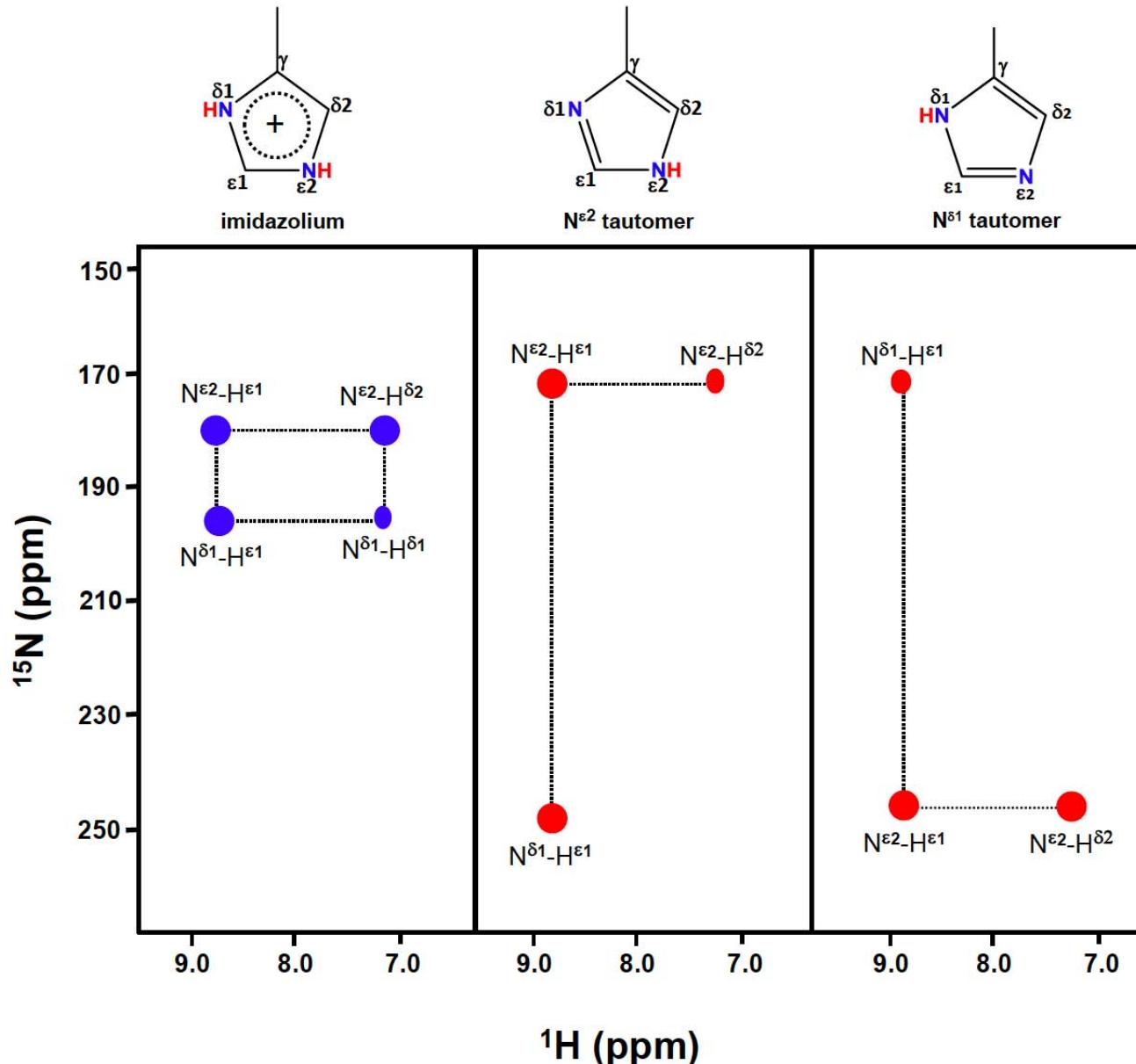
How does lysine dynamics influence GAG binding – NMR studies

Table 1 Summary of CXCL1 and CXCL5 lysine interactions with heparin

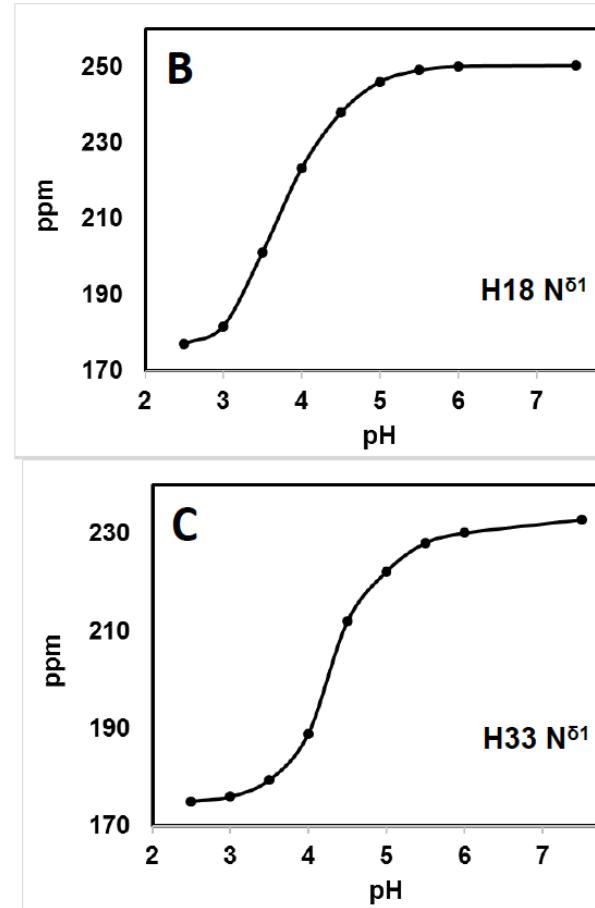
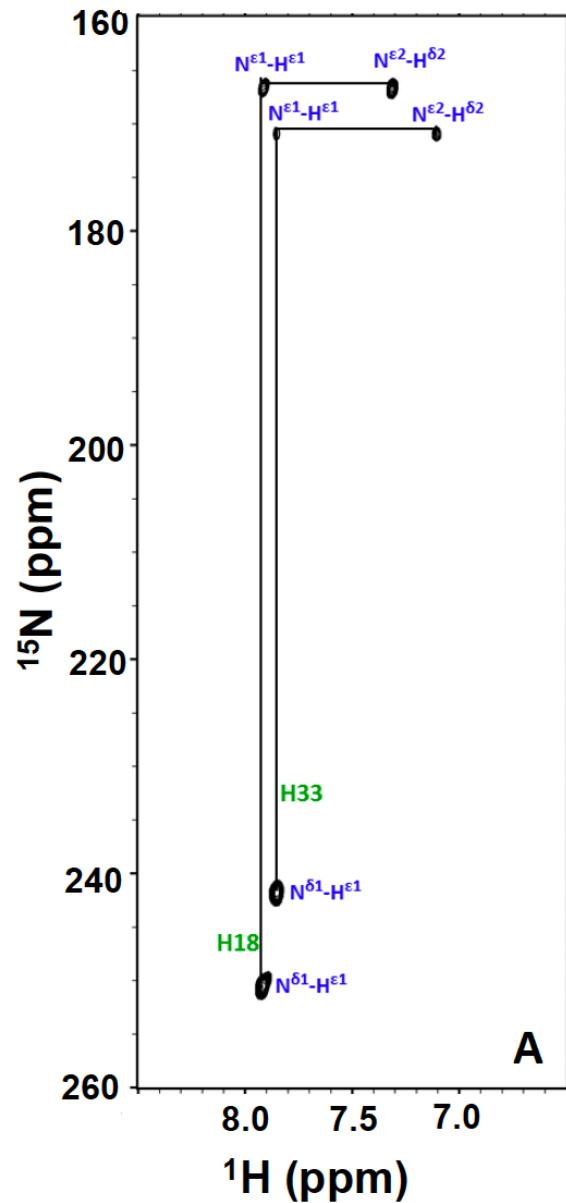
CXCL1	K21	K29	K45	K49	K60	K61	K65	K71
dp14								
HISQC	Y	Y	Y	Y	Y	Y	Y	N
H2CN	Y	Y	Y	Y	Y	Y	Y	N
HSQC	Y	N	Y	Y	Y	Y	Y	N
dp8								
HISQC	Y	Y	Y	Y	Y	Y	Y	N
H2CN	Y	Y	Y	Y	Y	Y	Y	N
HSQC	Y	N	Y	Y	Y	Y	Y	N
CXCL5	K25	K41	K49	K52	K64	K65	K69	K76
dp14								
HISQC	Y	Y	Y	Y	Y	Y	Y	N
H2CN	Y	N	Y	Y	Y	Y	Y	N
HSQC	Y	N	Y	Y	Y	Y	Y	Y
dp8								
HISQC	Y	Y	Y	Y	Y	Y	Y	N
H2CN	Y	N	Y	Y	Y	Y	Y	N
HSQC	Y	N	Y	Y	Y	Y	Y	Y

HISQC (“Y” and “N” correspond to the presence or absence of a peak). H2CN (“Y” and “N” correspond to the presence or absence of a chemical shift perturbation). HSQC (“Y” and “N” correspond to the presence or absence of chemical shift perturbation).

Role of histidines in GAG binding – NMR studies

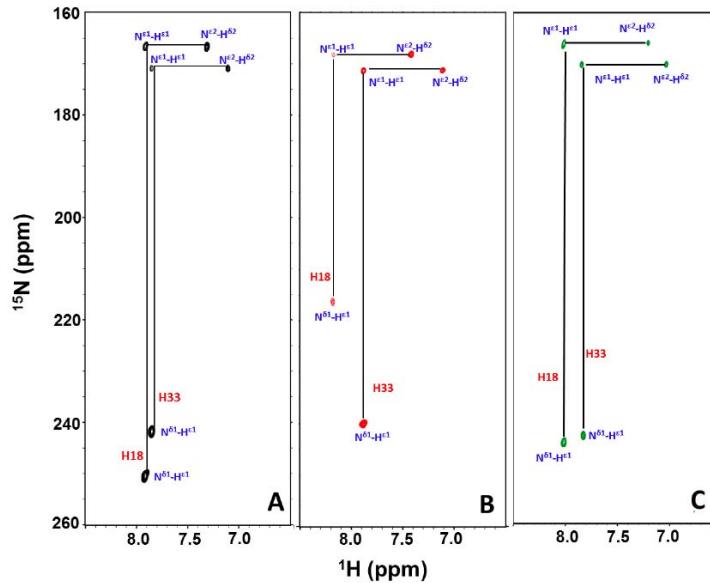


Role of histidines in GAG binding – NMR studies

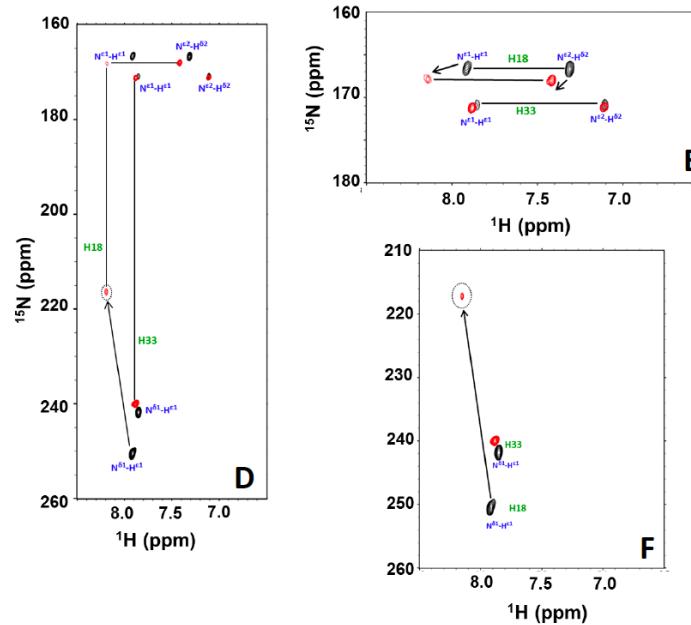


CXCL8 - Two conserved histidines
adopt the same ($\text{N}\varepsilon^2$) conformation

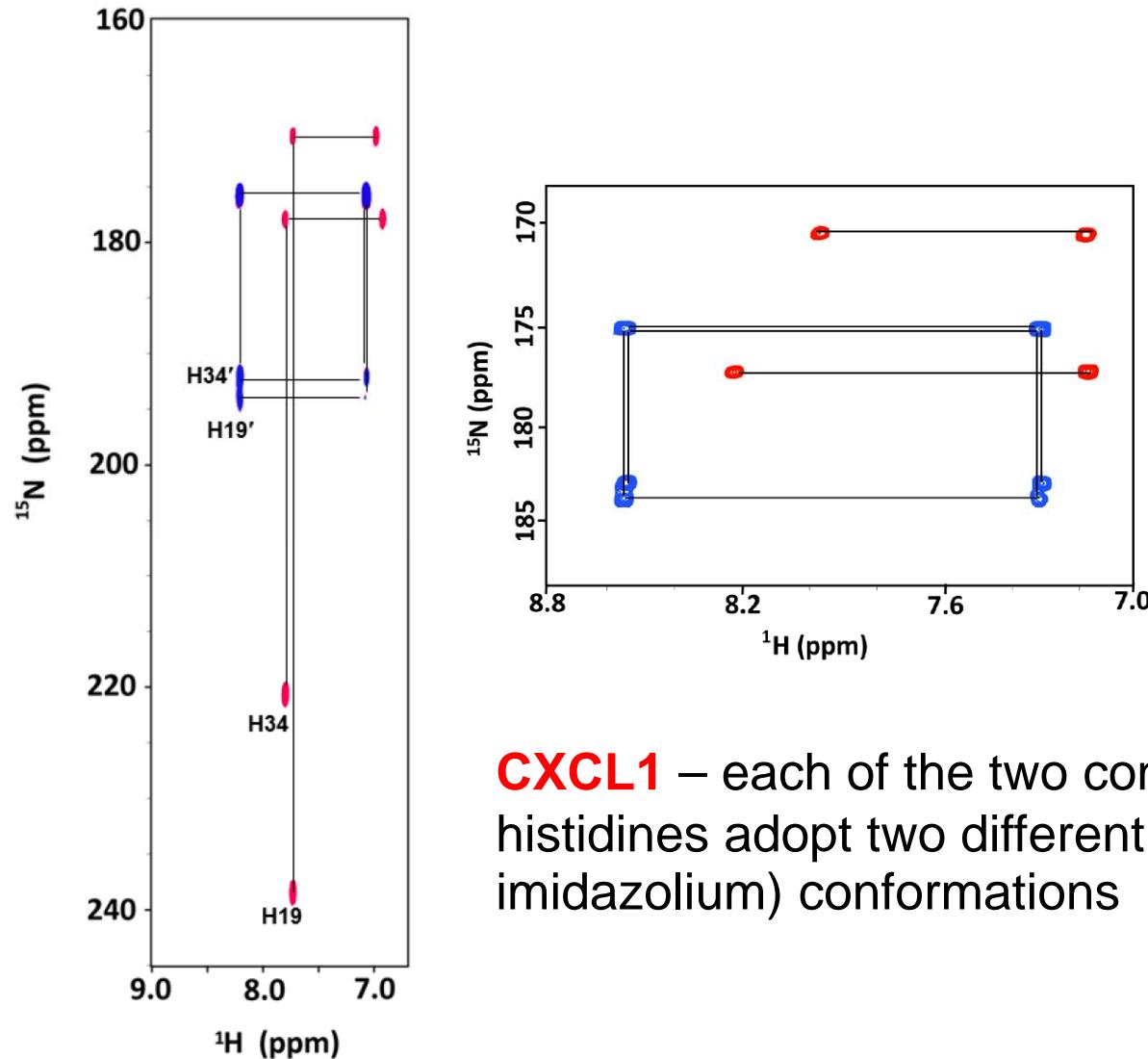
Role of histidines in GAG binding – NMR studies



(D to F) differences in free vs. bound spectra

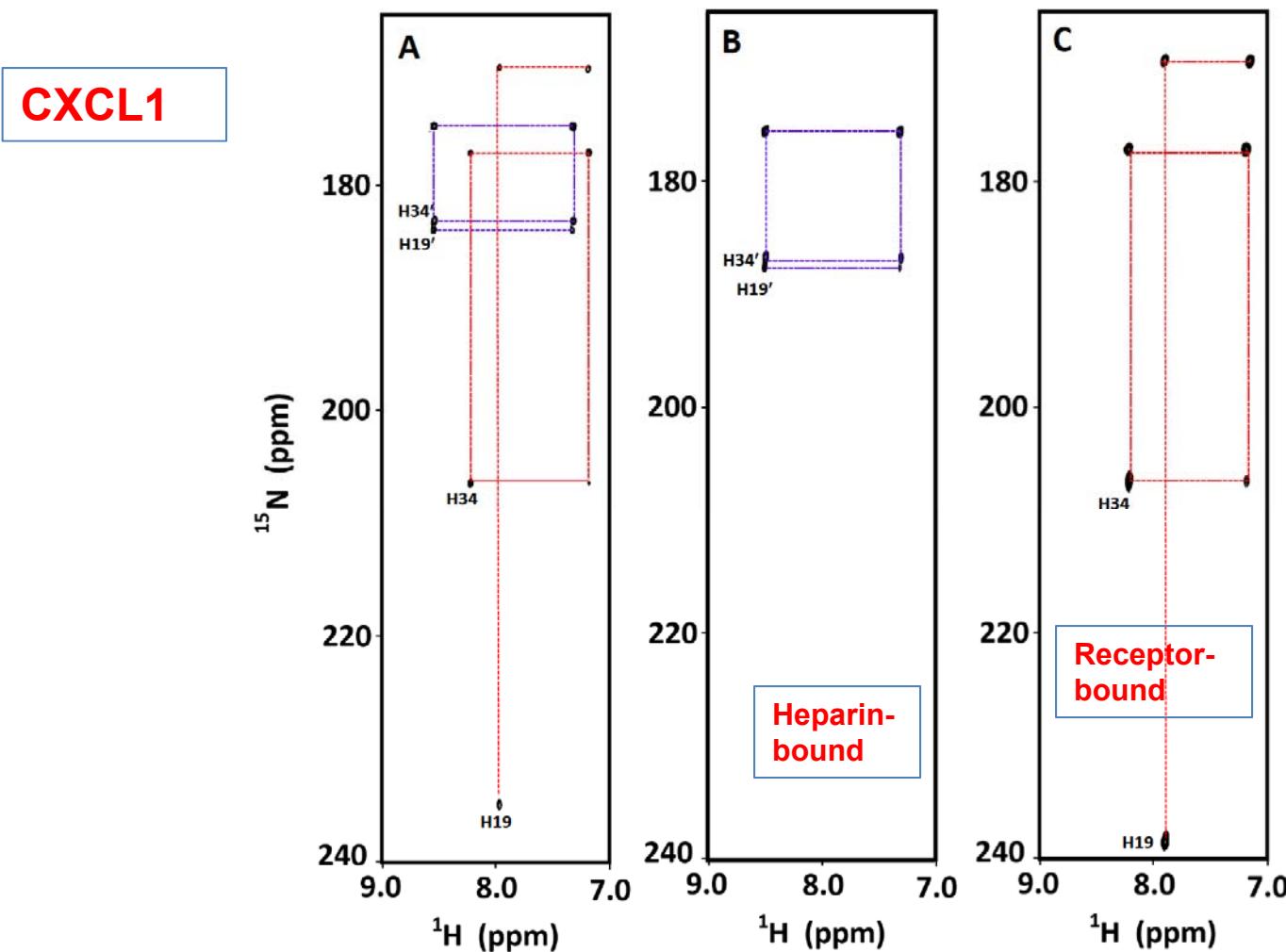


Role of histidines in GAG binding – NMR studies

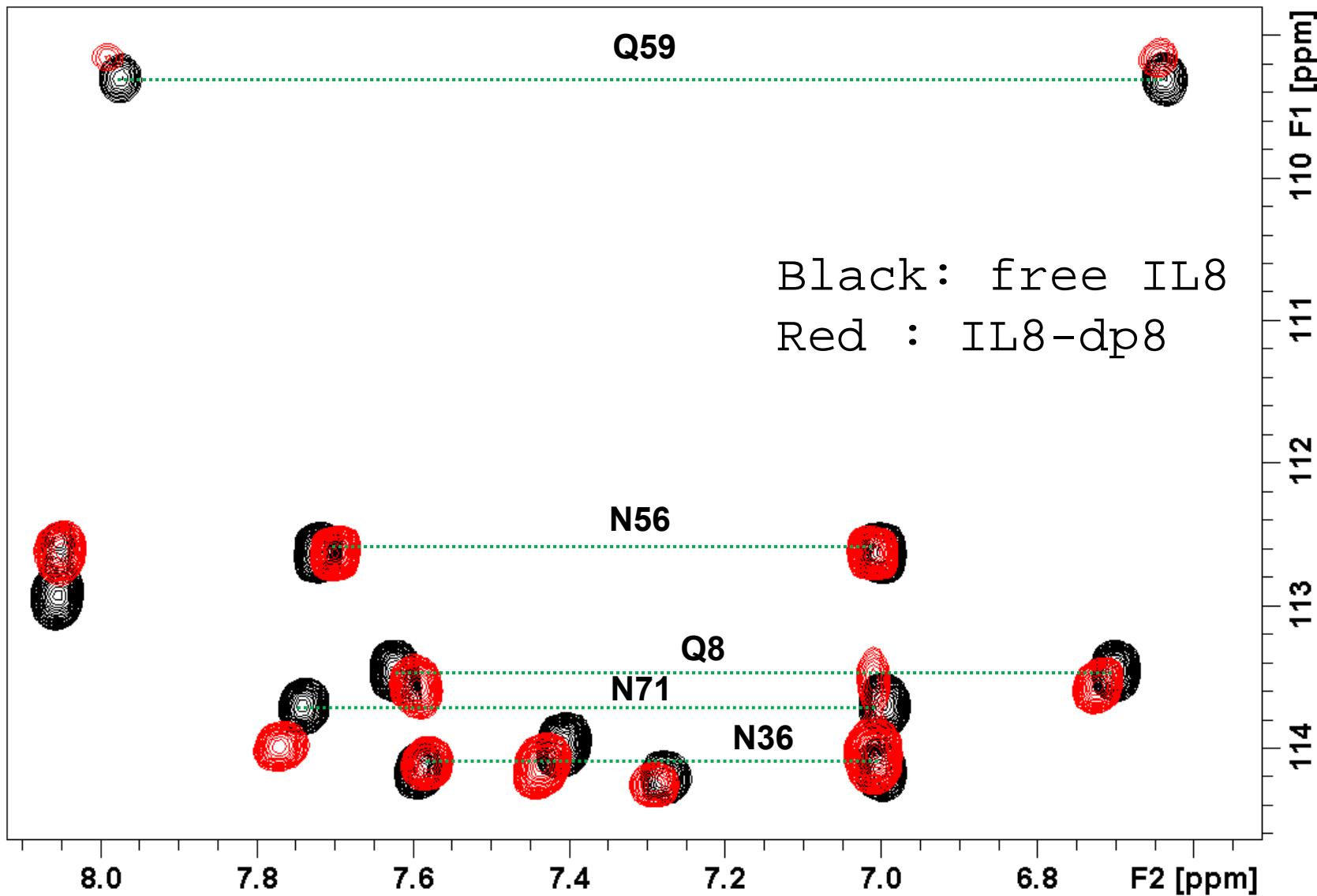


CXCL1 – each of the two conserved histidines adopt two different ($\text{N}\varepsilon 2$ and imidazolium) conformations

Role of histidines in GAG binding – NMR studies



Role of Asn/Gln in GAG binding – NMR studies



Role of Asn/Gln in GAG binding – NMR studies

