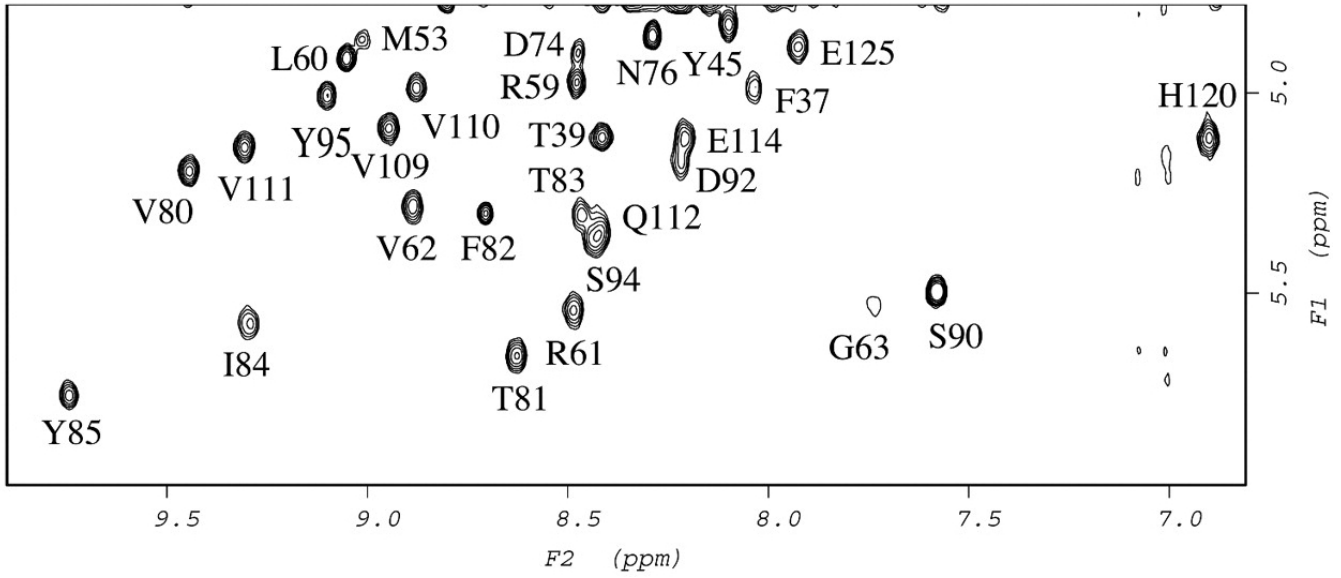
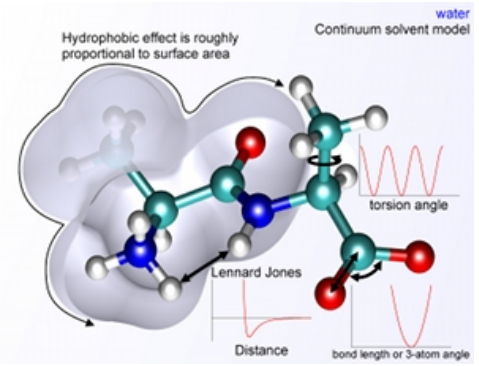
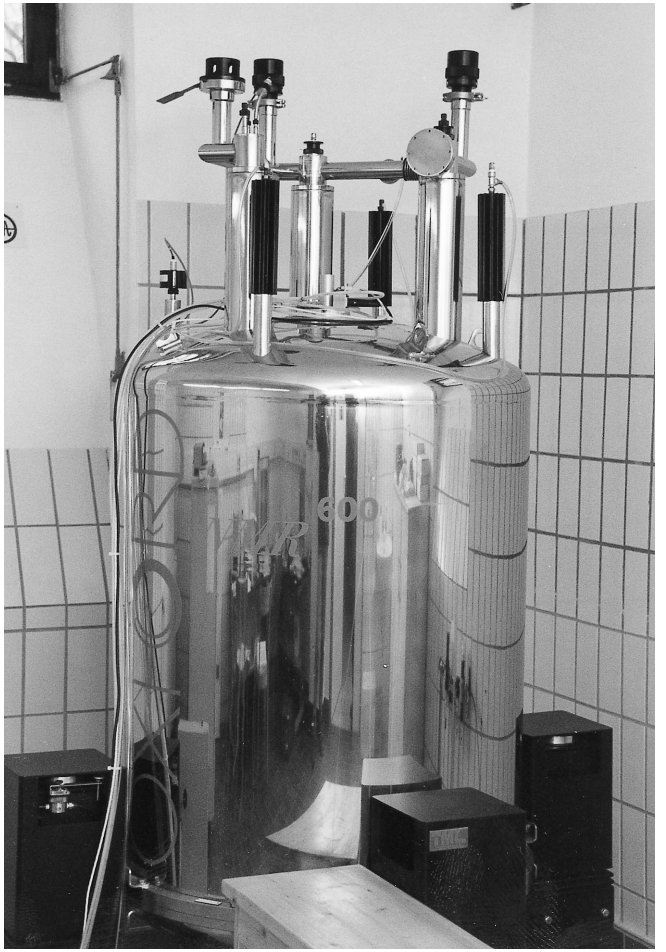
Wintersemester 2015/2016
Biomolecular Engineering/Nanobiophysics Module

LECTURE 7: QM, MD AND NMR



$$H(t)|\psi(t)\rangle = i\hbar\frac{d}{dt}|\psi(t)\rangle$$



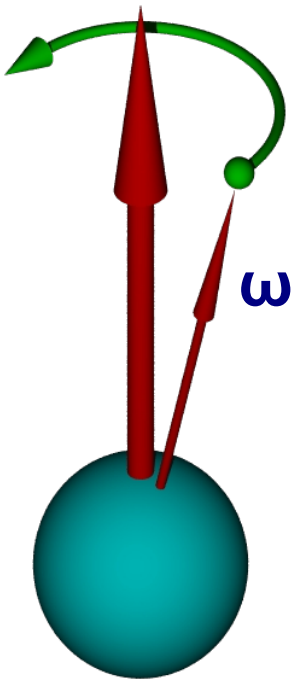


LECTURE 7: QM, MD AND NMR

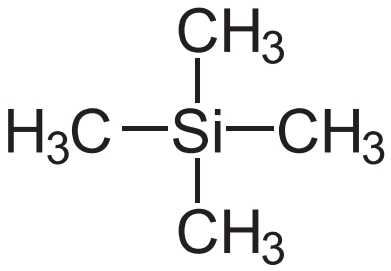
- Basics of NMR
- NMR and QM: GIAO method
- NMR and MD: Karplus equation
- Software for calculation NMR parameters
- Case study 1: GIAO calculations for saccharides
- Case study 2: IL-8 interactions with GAGs by NMR and MD

NMR EXPERIMENT

- Nuclear magnetic resonance is a physical phenomenon, in which magnetic field is absorbed and re-emitted by nuclei.

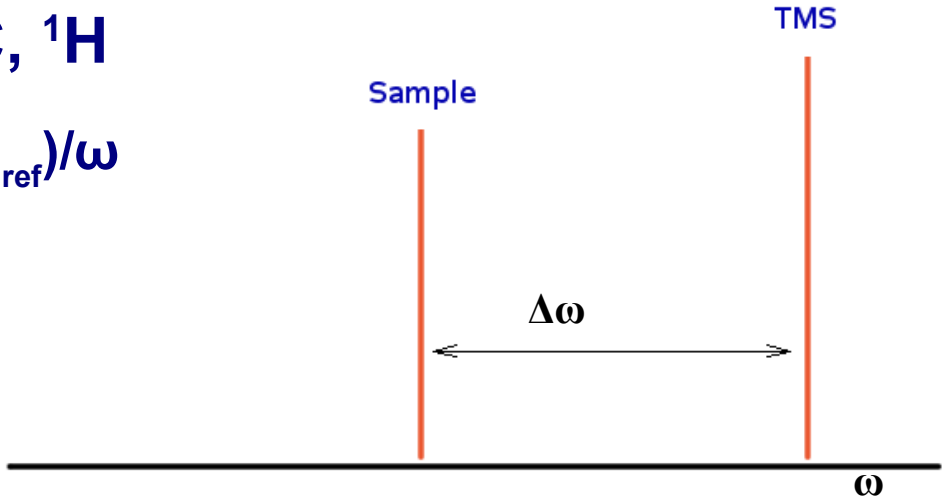


Chemical shift (Δppm): ^{13}C, ^{1}H



$$\Delta ppm=(\omega-\omega_{ref})/\omega$$

$$\Delta\omega=\omega-\omega_{ref}$$



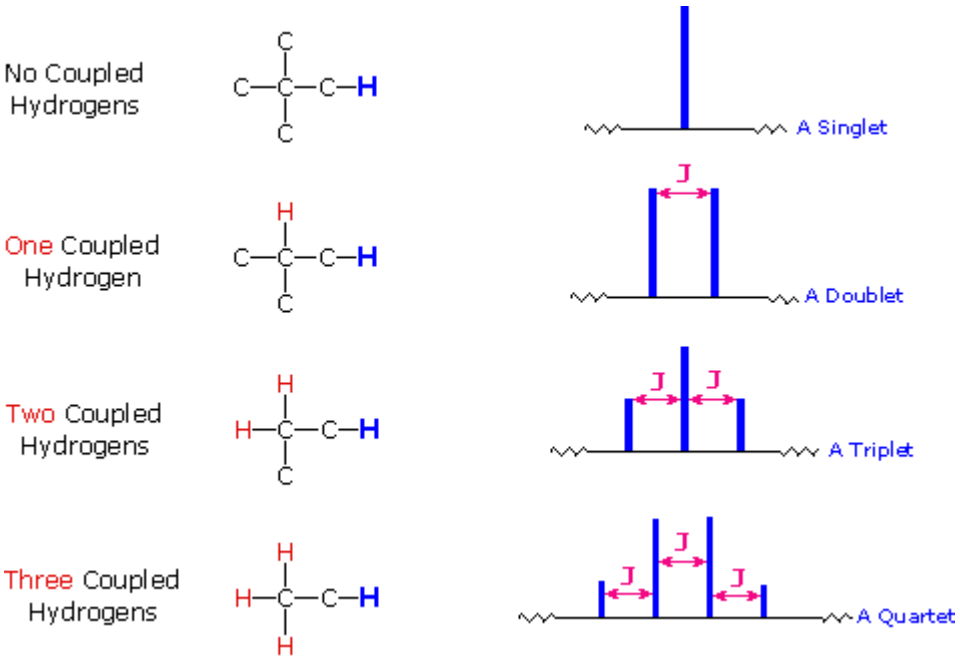
Reference: TMS (Tetramethylsilane)

J-couplings:

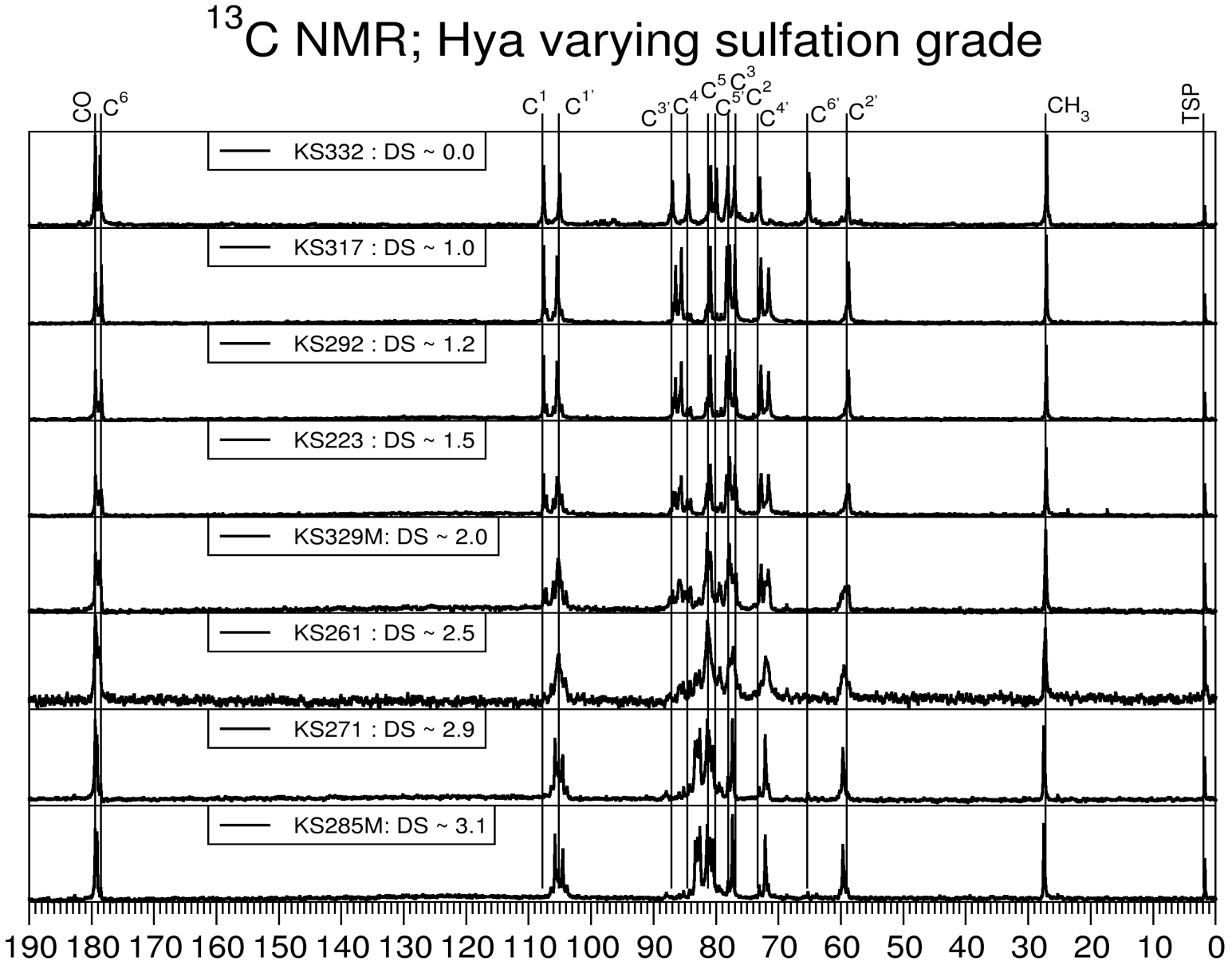
$$^{3}J_{H,H}(\varphi)=A\cos^2\varphi+B\cos\varphi+C$$

φ- dihedral

Magnetic interactions between nuclear spin and electron/nuclei spin around ~ chemical environment

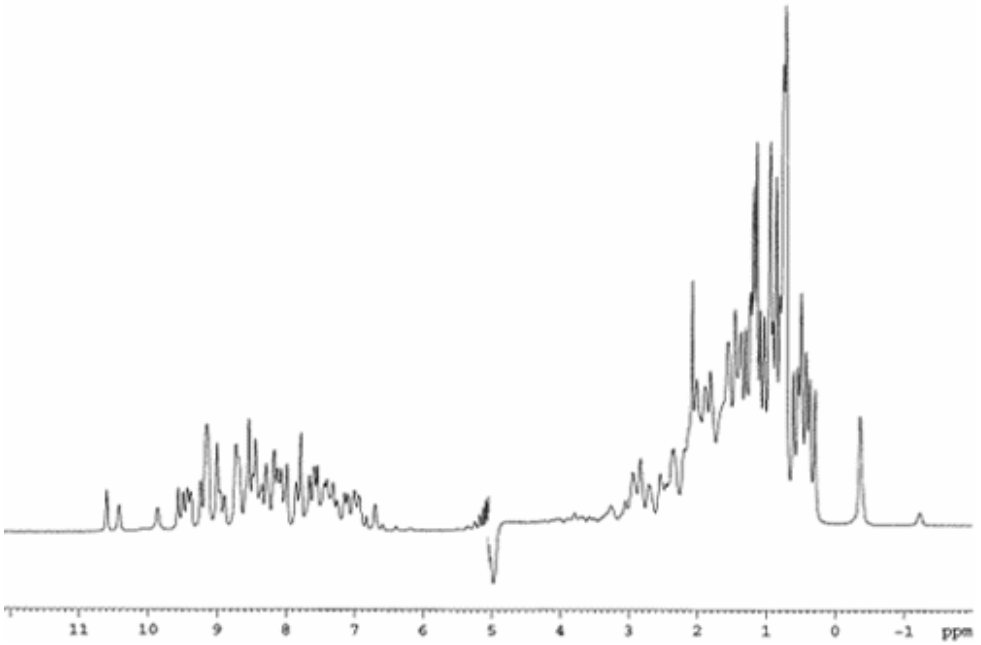


EXAMPLE: HA SULFATION IN NMR



NMR AND QM

- Chemical shifts of ^{1}H and ^{13}C
- J-couplings (^{1}H-^{1}H, ^{13}C-^{1}H and ^{13}C-^{13}C)
- Spectrum is known but peaks are not assigned



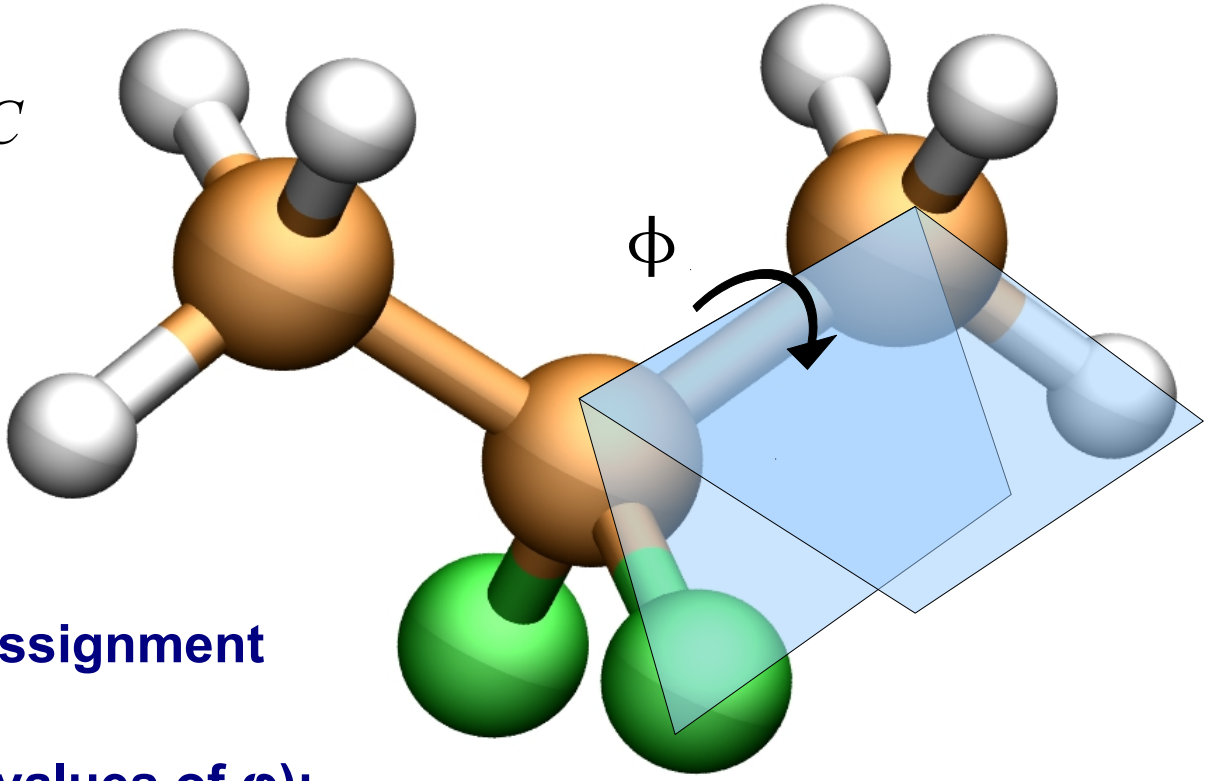
- Molecules geometry (HF, DFT)
- Energies *in vacuo*
- NMR parameters: chemical shifts and J-couplings (GIAO – *gauche independent atomic orbitals*)

$$\vec{M}_{ijk} = \text{invariant (x, y, z)}$$

- Parameters are assigned to each atom

NMR AND MD: KARPLUS EQUATION

$$J(\phi) = A\cos^2(\phi) + B\cos(\phi) + C$$



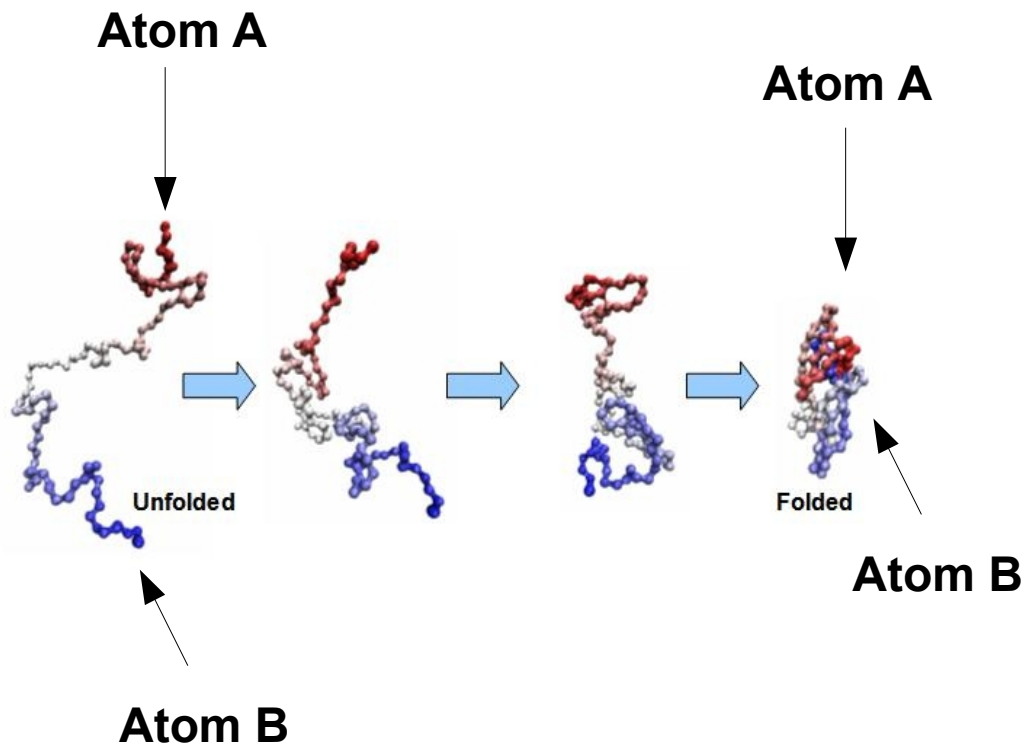
- Calibration of force fields
- Conformational studies
- Assistance by NMR spectra assignment
- Example (3 conformations, 3 values of φ):

$$J(\phi) = C_1 J(\phi_1) + C_2 J(\phi_2) + C_3 J(\phi_3)$$

- NMR: average value
- MD: all values with probabilities => C_1, C_2, C_3
- Comparison/Spectra analysis

NMR RESTRAINTS IN MD

- Initially used for refinement of NMR data with a force field
- Restraints in MD to be biased to NMR results (decrease confromational space) or to fix the studied conformation:
  - bonds
  - angles
  - torsions
  - *distance
  - *improper torsions
- Steered dynamics:
  - folding
  - conformational changes
  - binding of molecules

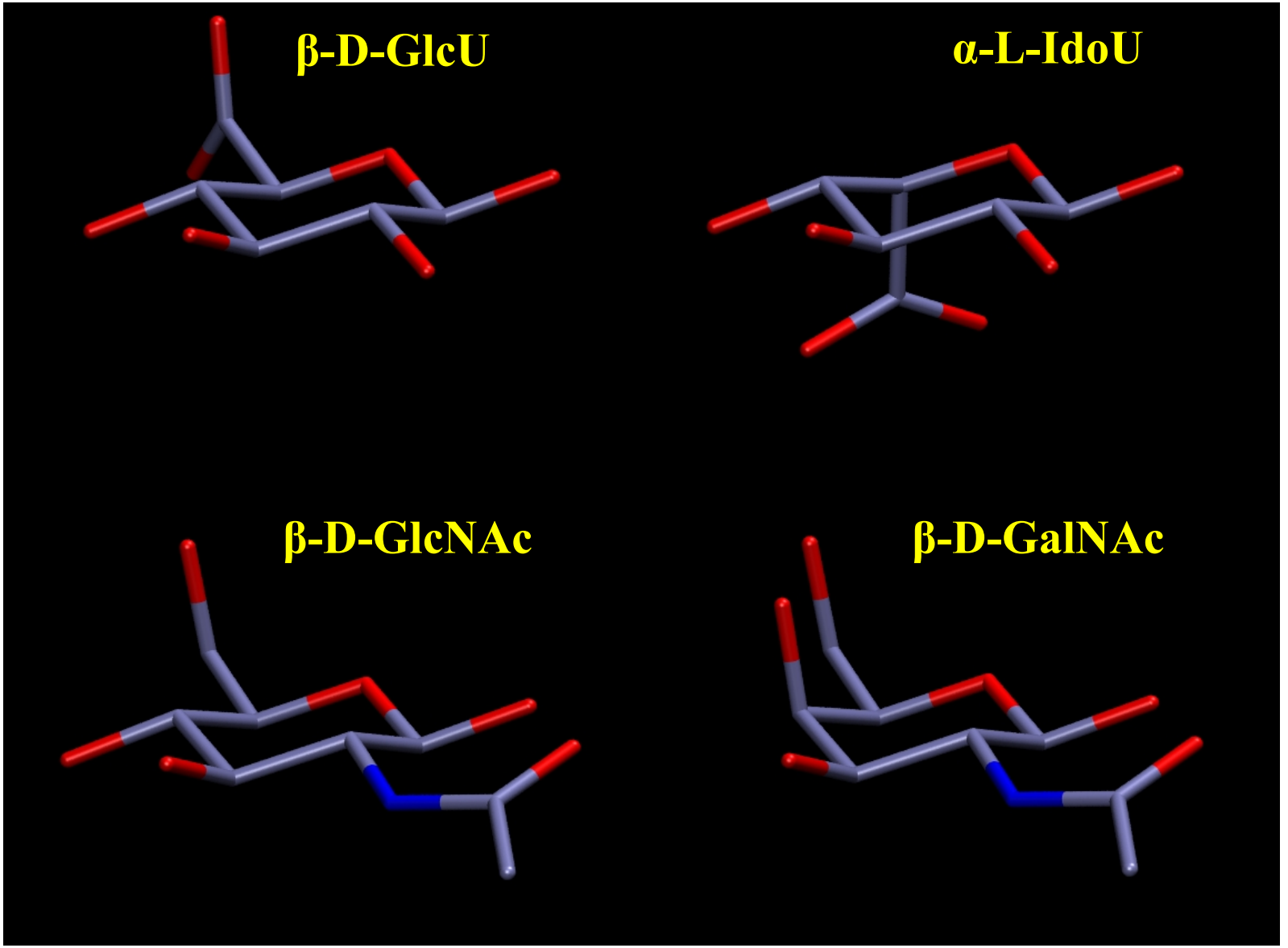


Parameters:
- speed (how many steps)
- force at each step ~k ($X-X_n$)

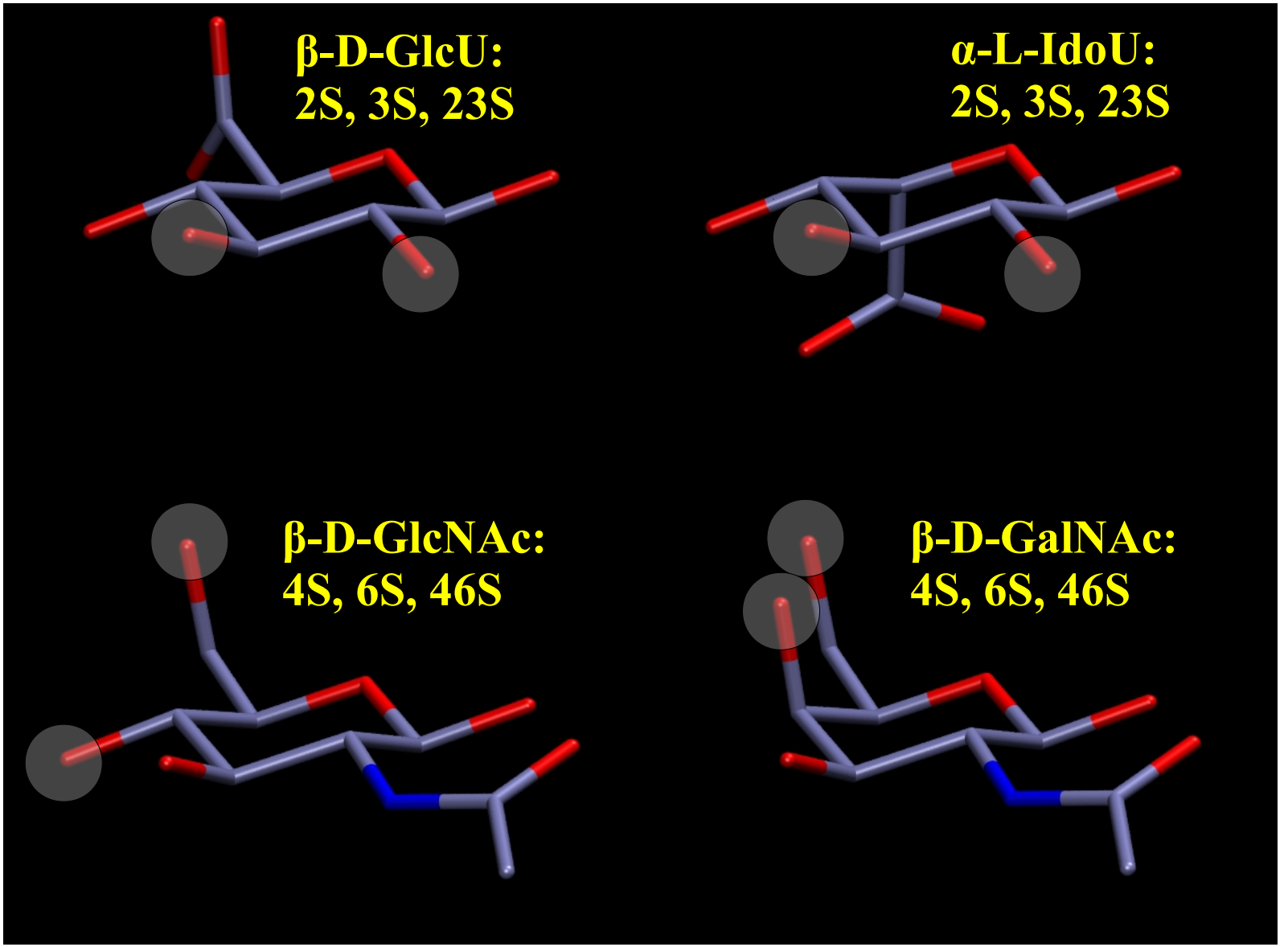
SOFTWARE

- GIAO: GAUSSIAN
- Absolute chemical shifts for proteins: CS-ROSETTA, SHIFTS, SHIFTX, SPARTA, etc.
  - statistical (empirical) source of data
  - force field and accessible surface area principles
- Absolute chemical shifts for other molecules: NO
- Changes of chemical shifts: NO
- J-couplings: from MD

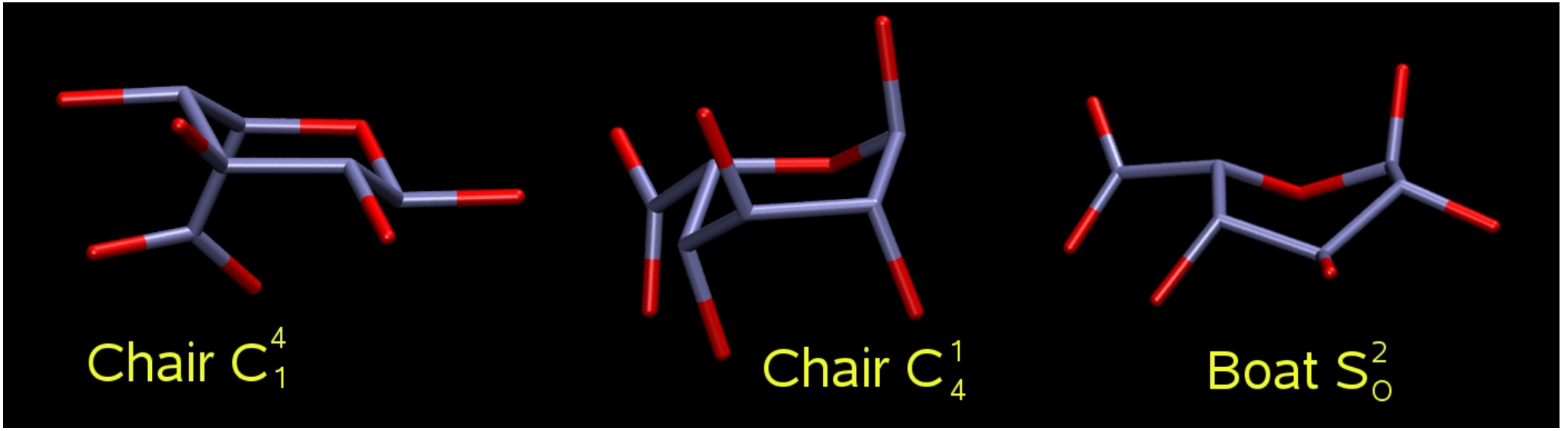
CASE STUDY 1:
QM CALCULATIONS OF NMR PARAMETERS FOR GAGS MONOSACCHARIDE-COMPONENTS



MONOSACCHARIDES IN GAGS: SULFATION



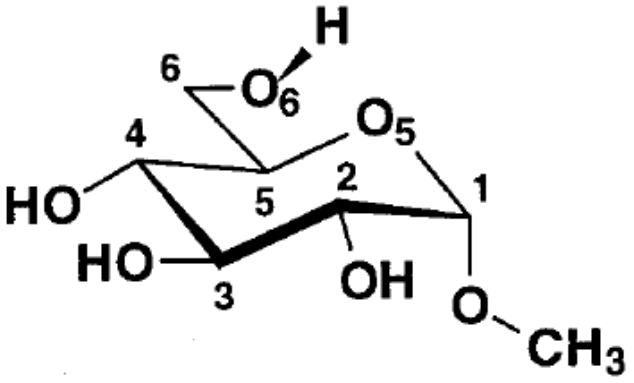
RING CONFORMATIONS



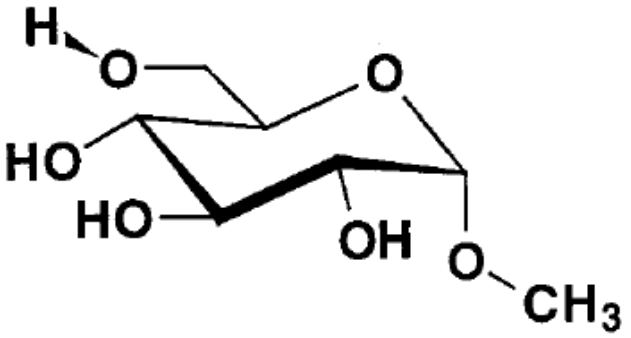
Chair C_1^4	Chair C_4^1	Boat S_O^2
α-L-IdoU, α-L-IdoU(2S), β-D-GlcU, β-D-GlcNAc, β-D-GalNAc	α-L-IdoU, α-L-IdoU(2S)	α-L-IdoU, α-L-IdoU(2S)

- Ring conformation changes in ~ ms, not realistic for MD
- Influence of sulfation is unknown
- Solvent/ions influence is crucial
- NMR and QM detect differences (Δppm, J-couplings)

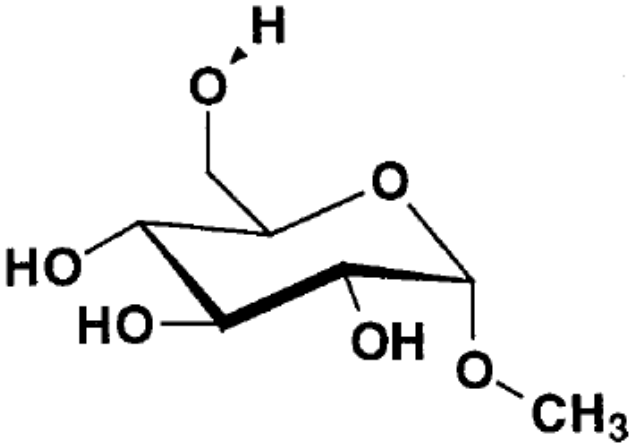
C5-C6: GG/GT/TG



gt (ω = ~60°)



tg (ω = ~180°)

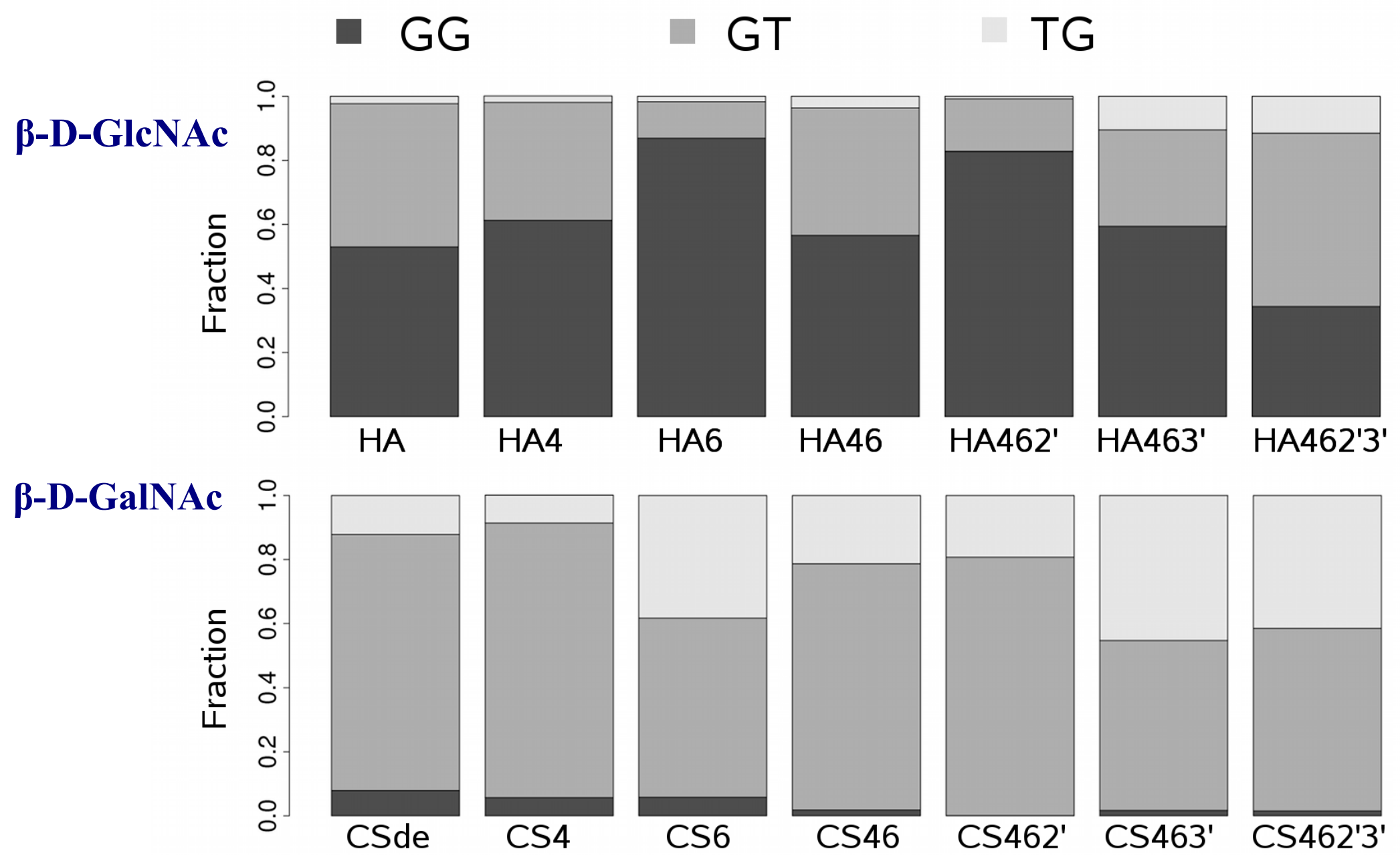


gg (ω = ~300°)

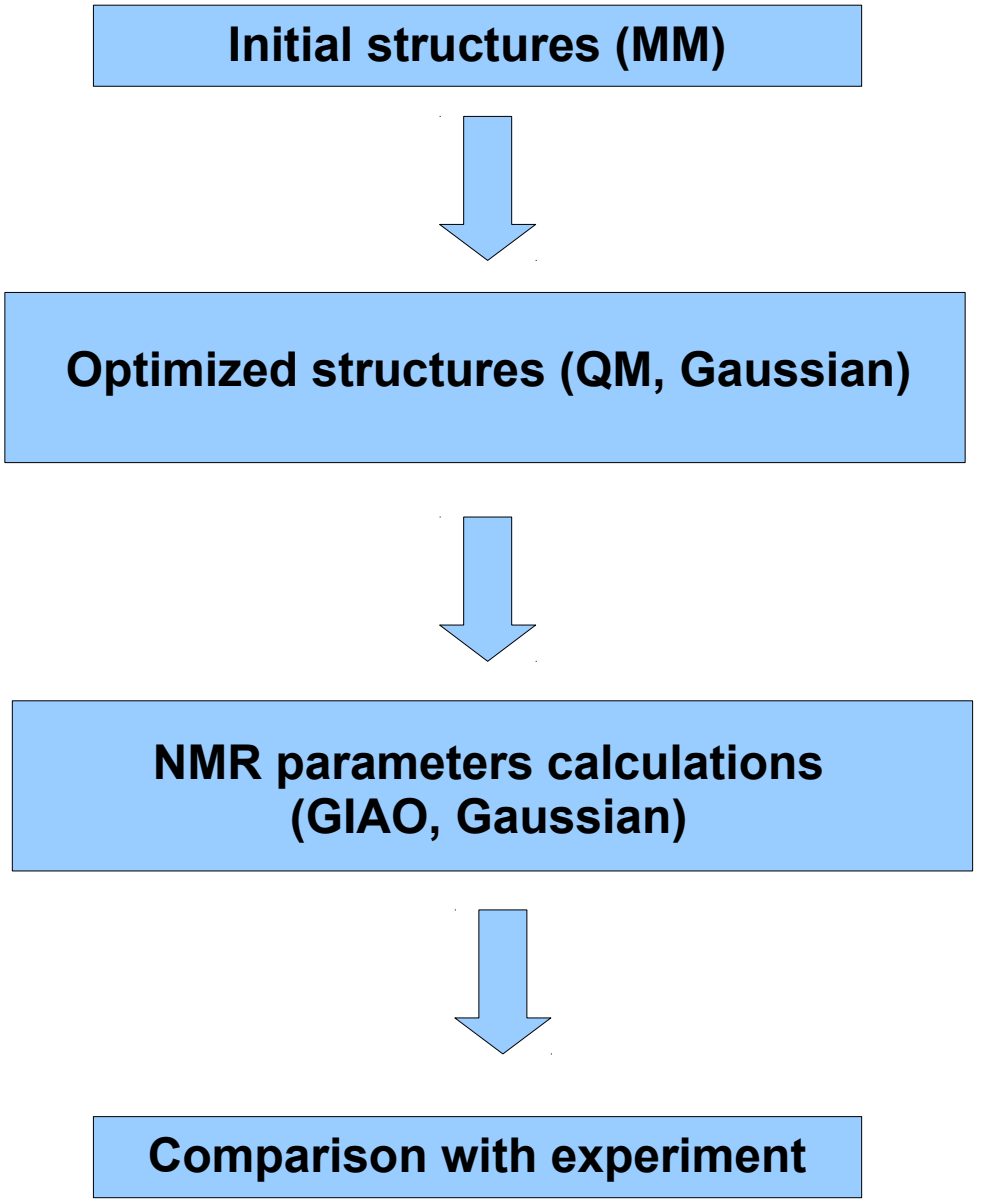
ω=dihedral(C4, C5, C6, O6)

- gg/gt/tg changes in ~ ps, realistic in MD
- Influence of sulfation is unknown
- Solvent/ions influence is crucial
- NMR and QM detect differences (J-couplings)

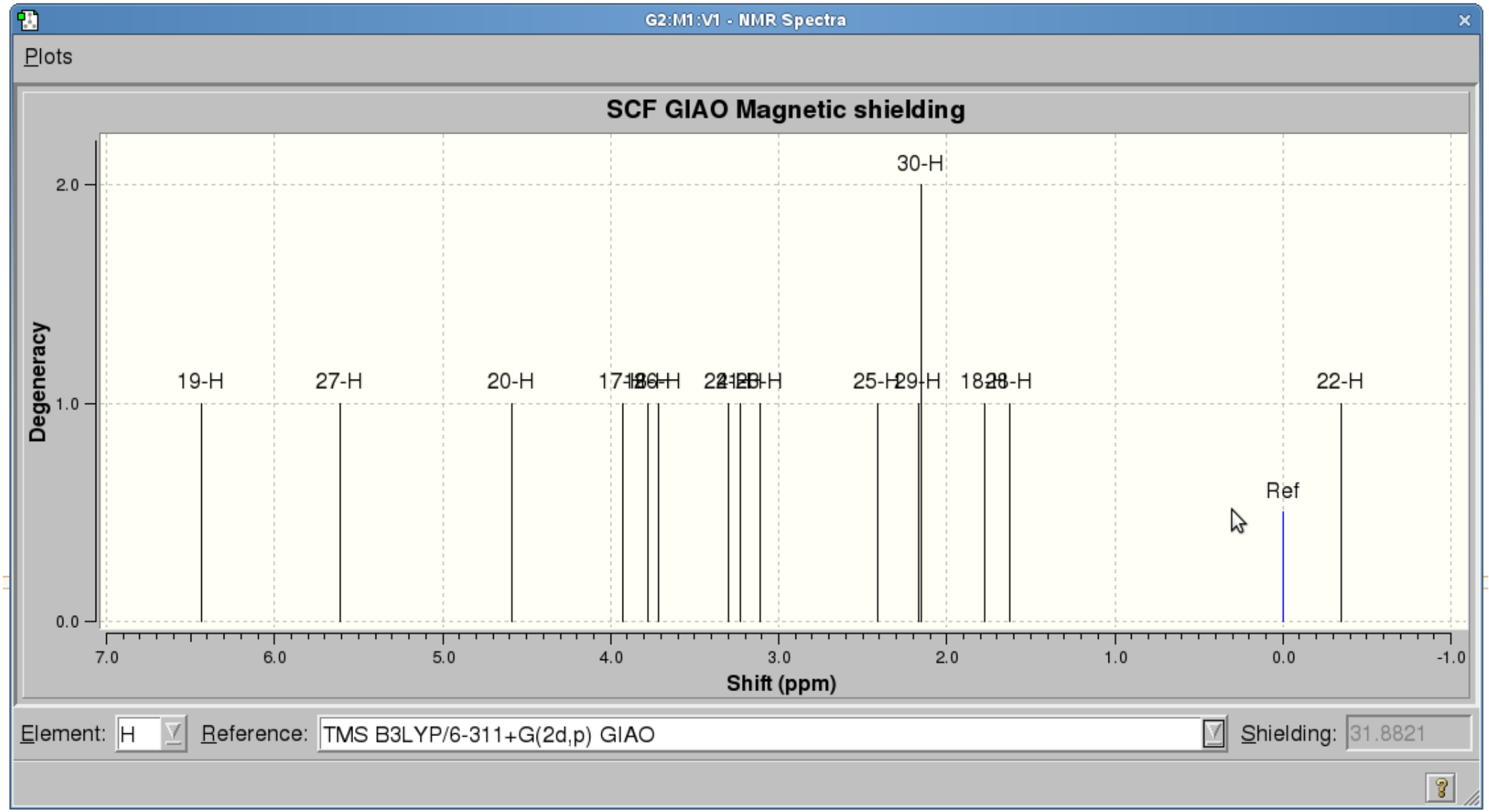
MD (HEXAGAGS, 20 ns): GG/GT/TG



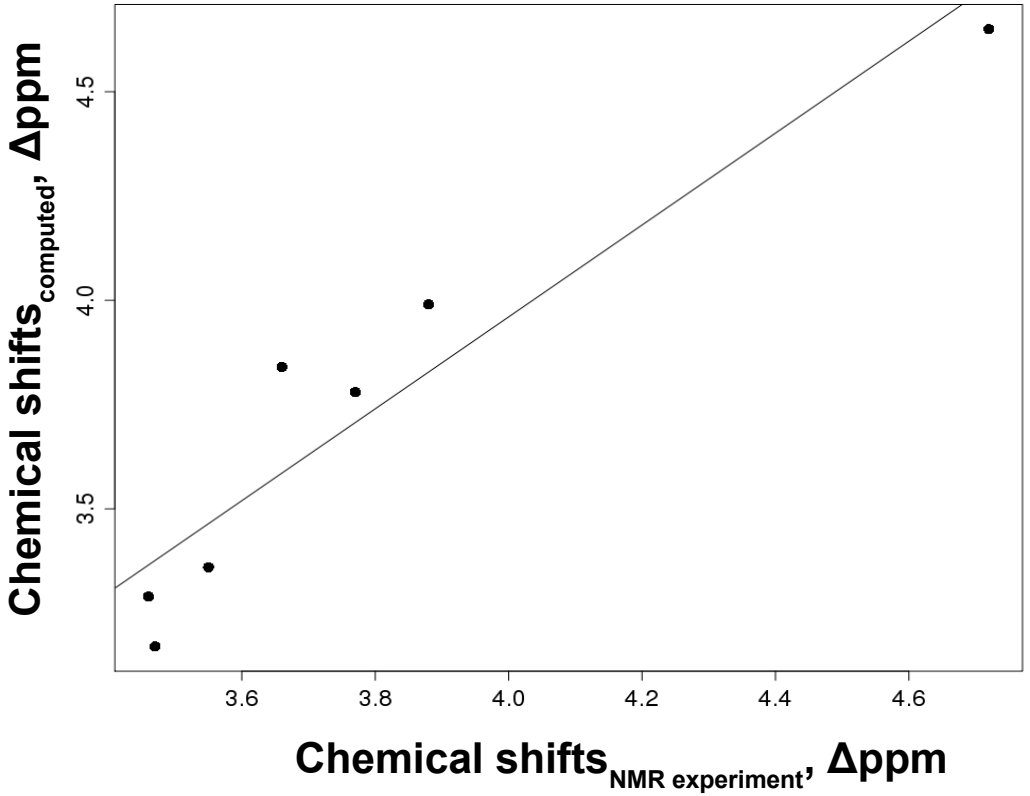
QM METHODOLOGY FOR NMR PARAMETERS

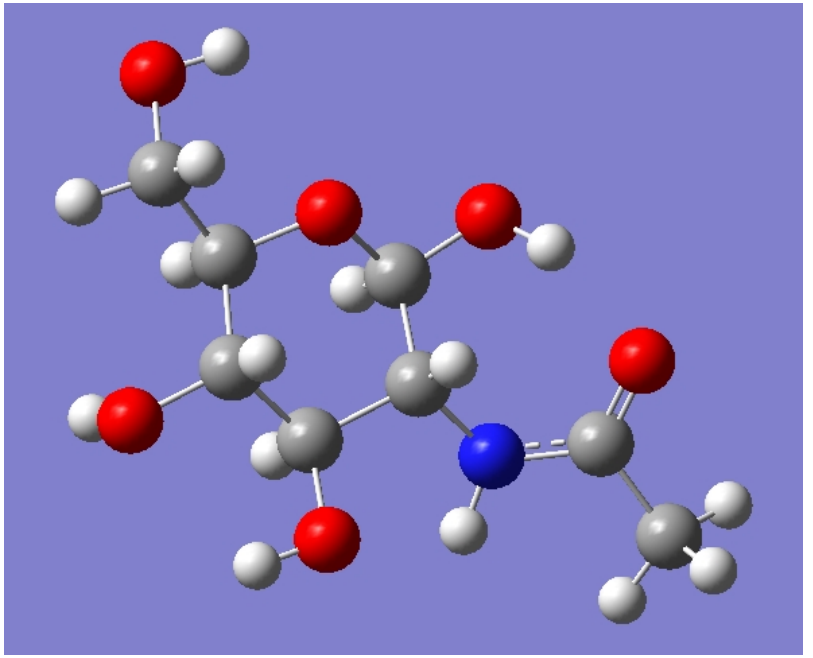


CALCULATED SPECTRUM IN GAUSSIAN



COMPARISON WITH THE EXPERIMENT





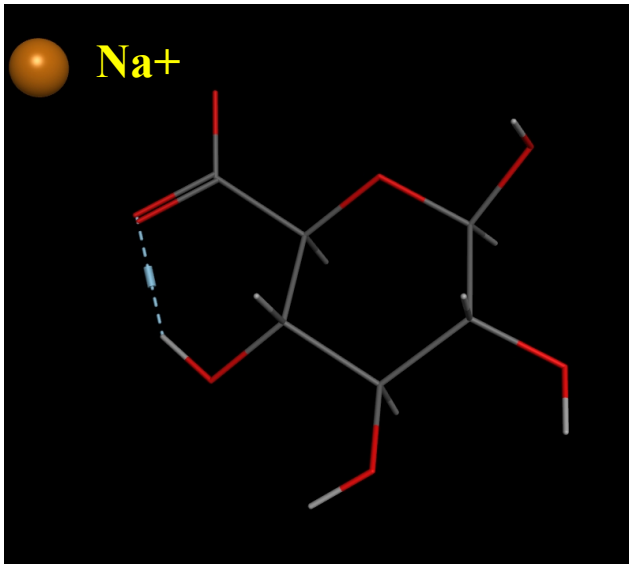
β-D-GlcNAc: C^4_1, GT-rotamer

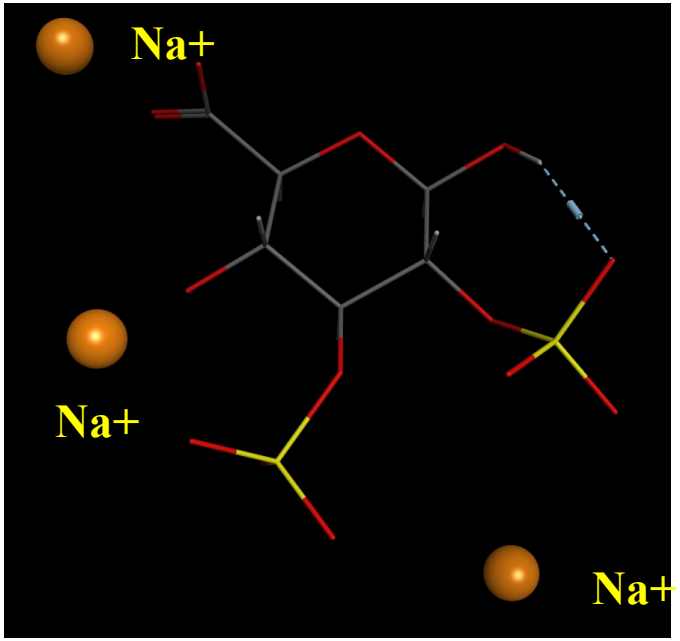
	Chemical shifts, Δppm B3LYP/6-311G(2d,p)	J-couplings, Hz B3LYP/aug-cc-pVDZ)
Average mean error	0.15	1.79
Pearson correlation	0.95	0.89
Spearman correlation	0.93	0.79

- Intercept and slope: space for improvement

ENERGETICS

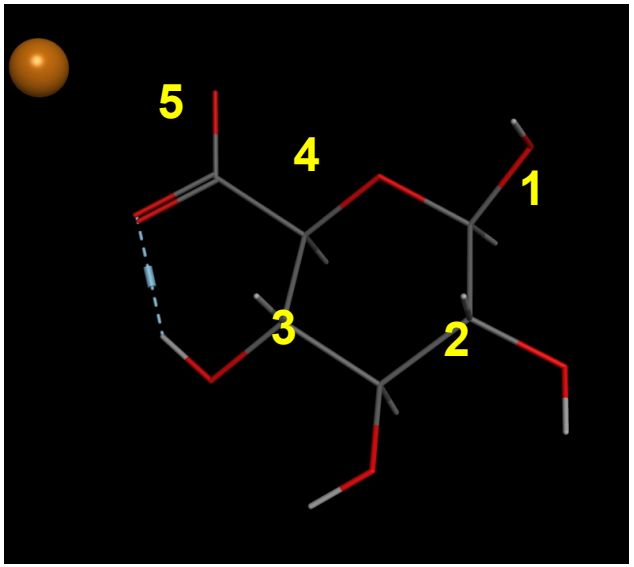
- Counterions are essential for these calculations:
  - electrostatics impact + another error introduction
  - agreement with previous works for Ido2S
- All 182 molecules/conformations are done:
  - Solvent in general decreases energy barriers
  - Methylation changes minimum for conformations in 7/16 cases *in vacuo* 5/16 cases in solvent
- For GlcNAc and GalNAc C^4_1 is preferred for all except 1 molecule; for Ido2S – C^1_4; for GlcU3S and GlcU23S - S^2_O
- NMR parameters can help choosing model

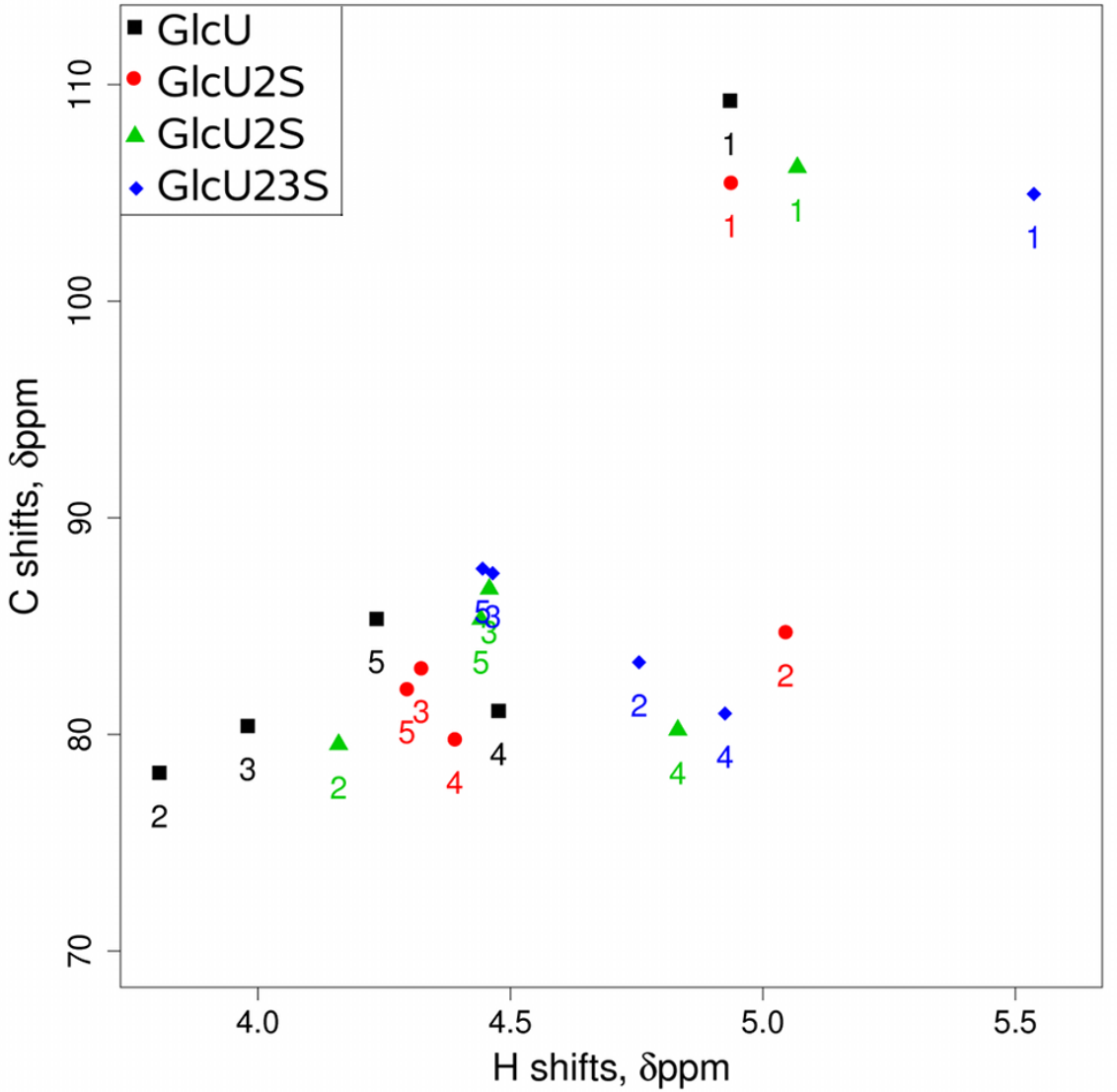




CHEMICAL SHIFTS

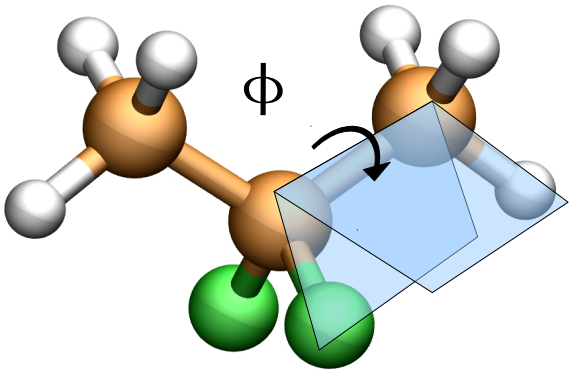
- Rings conformations do not contribute to chemical shifts
- Sulfation affects chemical shifts of:
  - Sulfated C
  - H bound to sulfated C
- Need for experiment to prove *significant* differences



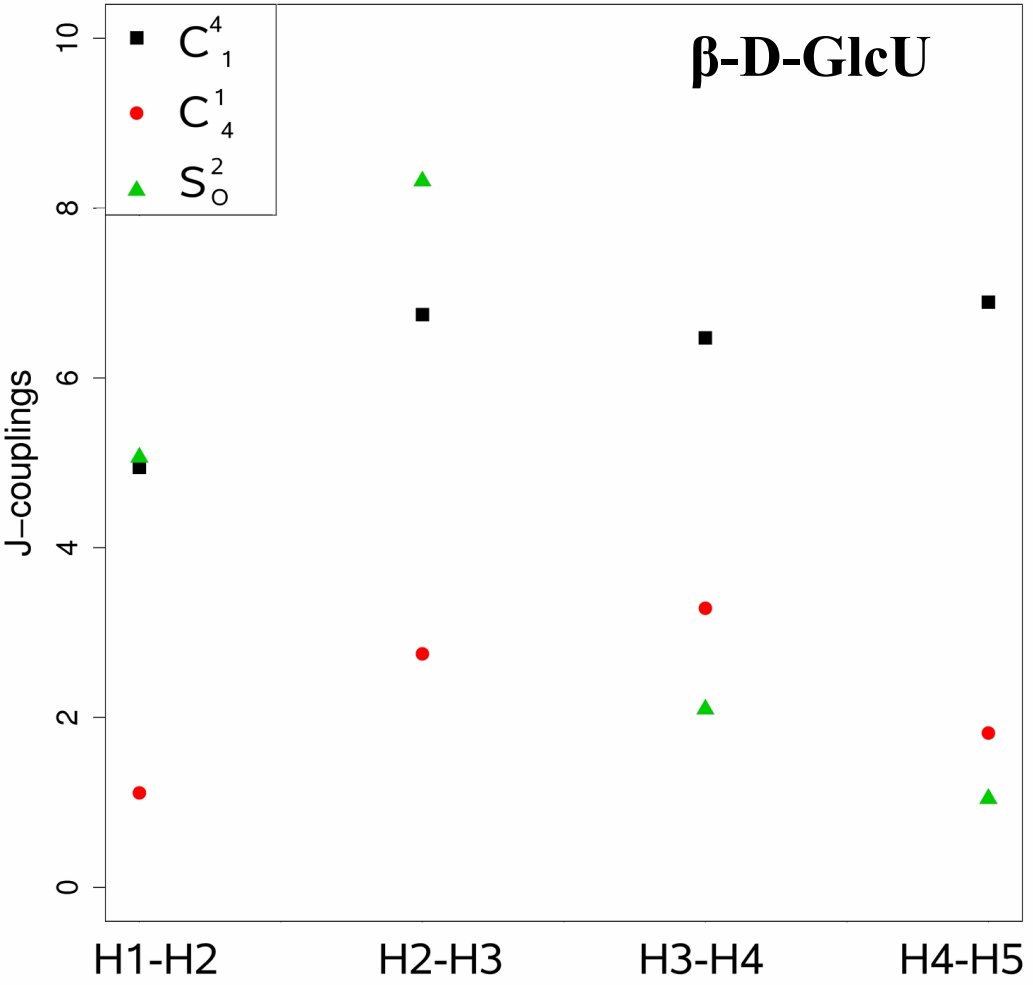


J-COUPLINGS

$$J(\phi) = A\cos^2(\phi) + B\cos(\phi) + C$$

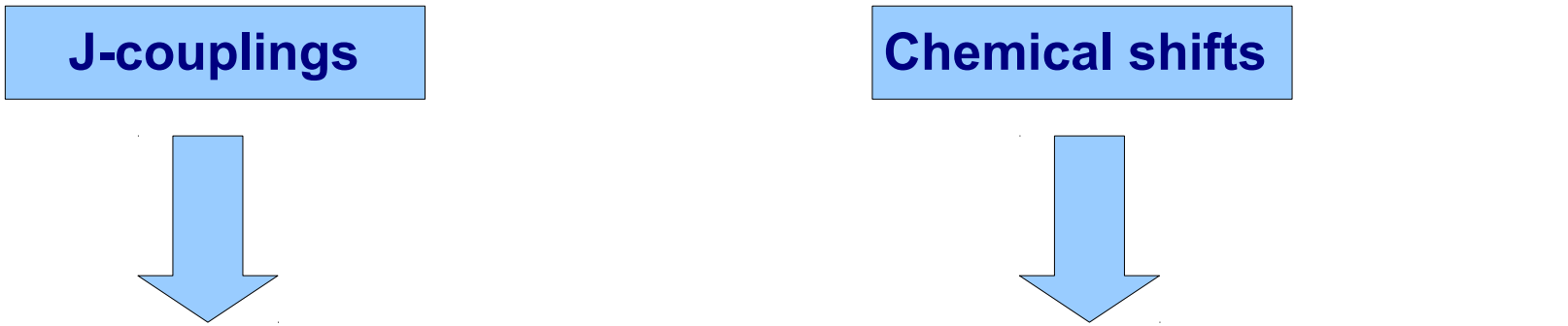


- **Rings conformations do clearly contribute to J-couplings**
- **Sulfation and methylation do not affect J-coupling**
- **Need for experiment to prove *significant* differences and define accuracy**



SUMMARY

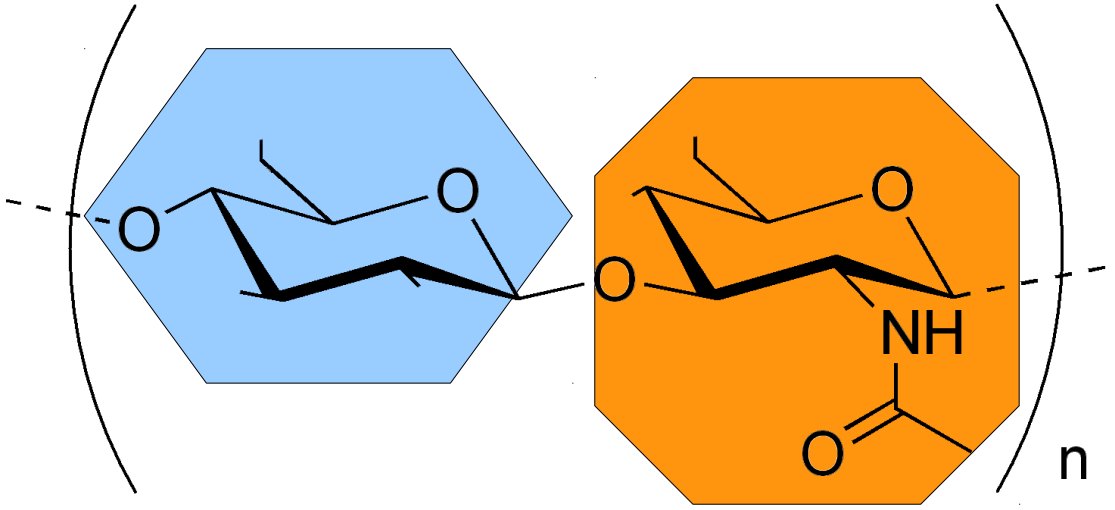
- Calculated chemical shifts and J-couplings well reproduce experimental values for GlcNAc
- J-couplings differ significanly for different ring conformations, whereas chemical shifts do not
- Chemical shifts reflect the pattern of sulfation whereas J-coupling do not
- Further experiments are needed



CASE STUDY 2

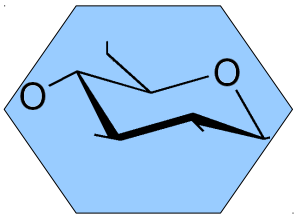
CHARACTERIZATION OF THE INTERACTION OF INTERLEUKIN-8 WITH HYALURONAN, CHONDROITIN SULFATE, DERMATAN SULFATE, AND THEIR SULFATED DERIVATIVES BY SPECTROSCOPY AND MOLECULAR MODELLING

GLYCOSAMINOGLYCANS (GAGs)



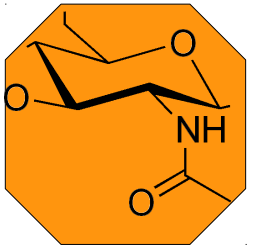
GAGs:

- Hyaluronan
- Chondroitin sulfate
- Heparin
- Heparan sulfate
- Keratan sulfate
- Dermatan sulfate



Hexose/Hexuronic acid:

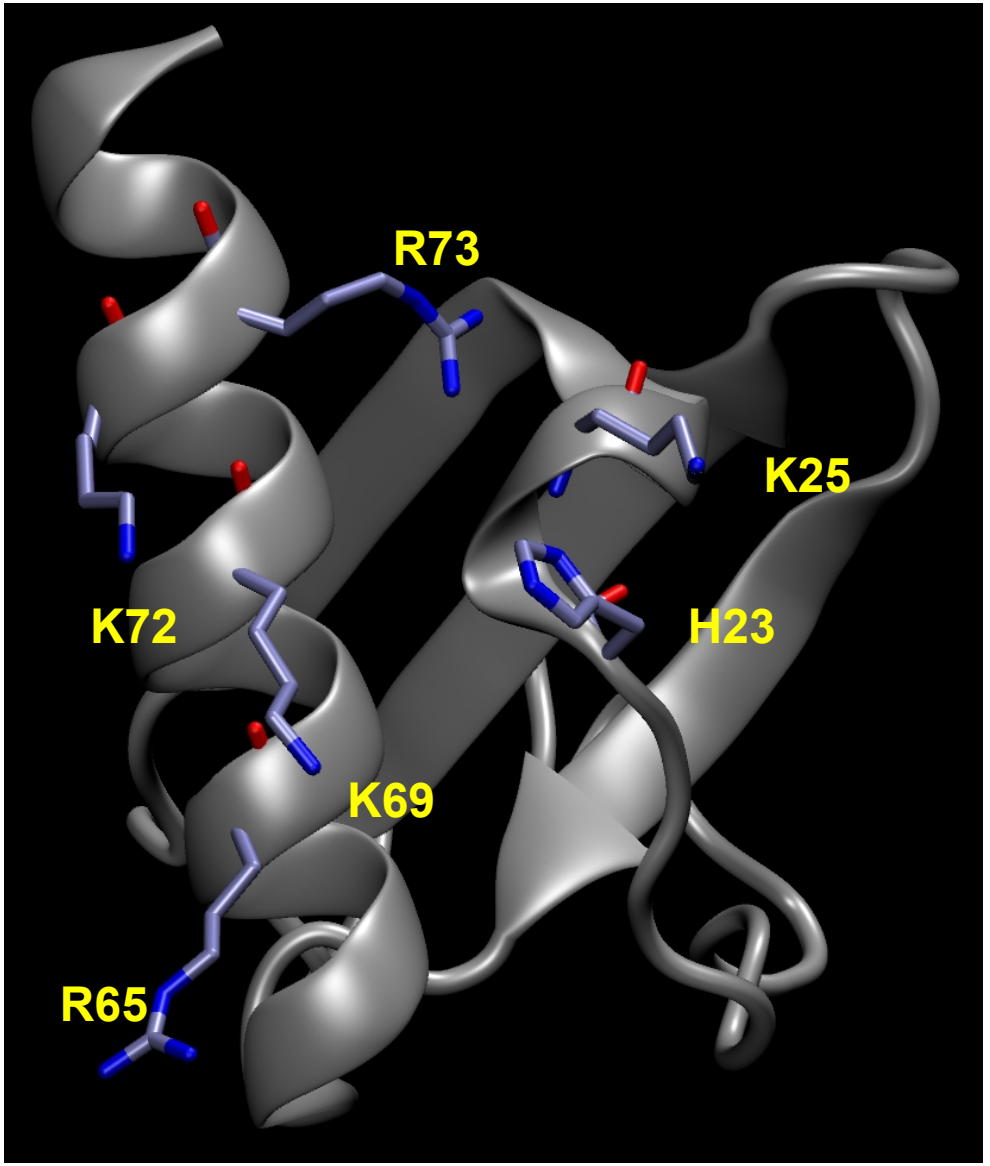
- GlcU
- IdoU
- Gal
- Sulfated derivatives



Hexosamine:

- GlcNAc
- GalNAc
- Sulfated derivatives

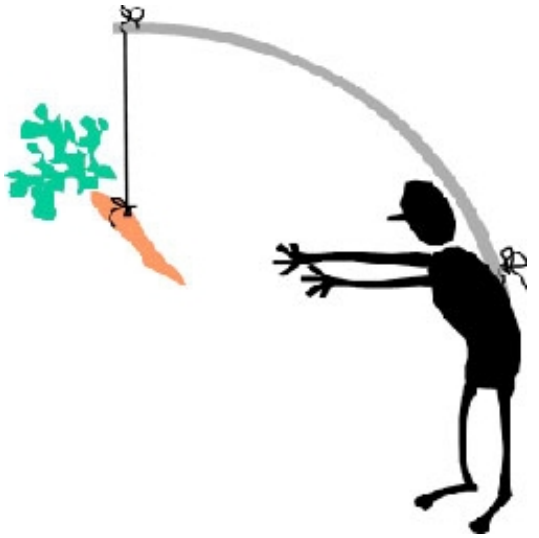
INTERLEUKIN-8



- IL-8 interaction with GAGs activates leukocytes
- IL-8 dimerization is influenced by GAGs binding
- Heparin binding site has been suggested by mutagenesis *(Kuschert et al. 1998)*

CHALLENGES AND MOTIVATION

UNKNOWN:



- Structures of IL-8 complexes with GAGs
- Quantitative impacts of individual IL-8 residues
- Specific binding for different GAGs or purely elect
- The size of essential GAG unit for IL-8 specific binding
- GAGs influence on IL-8 dimerization

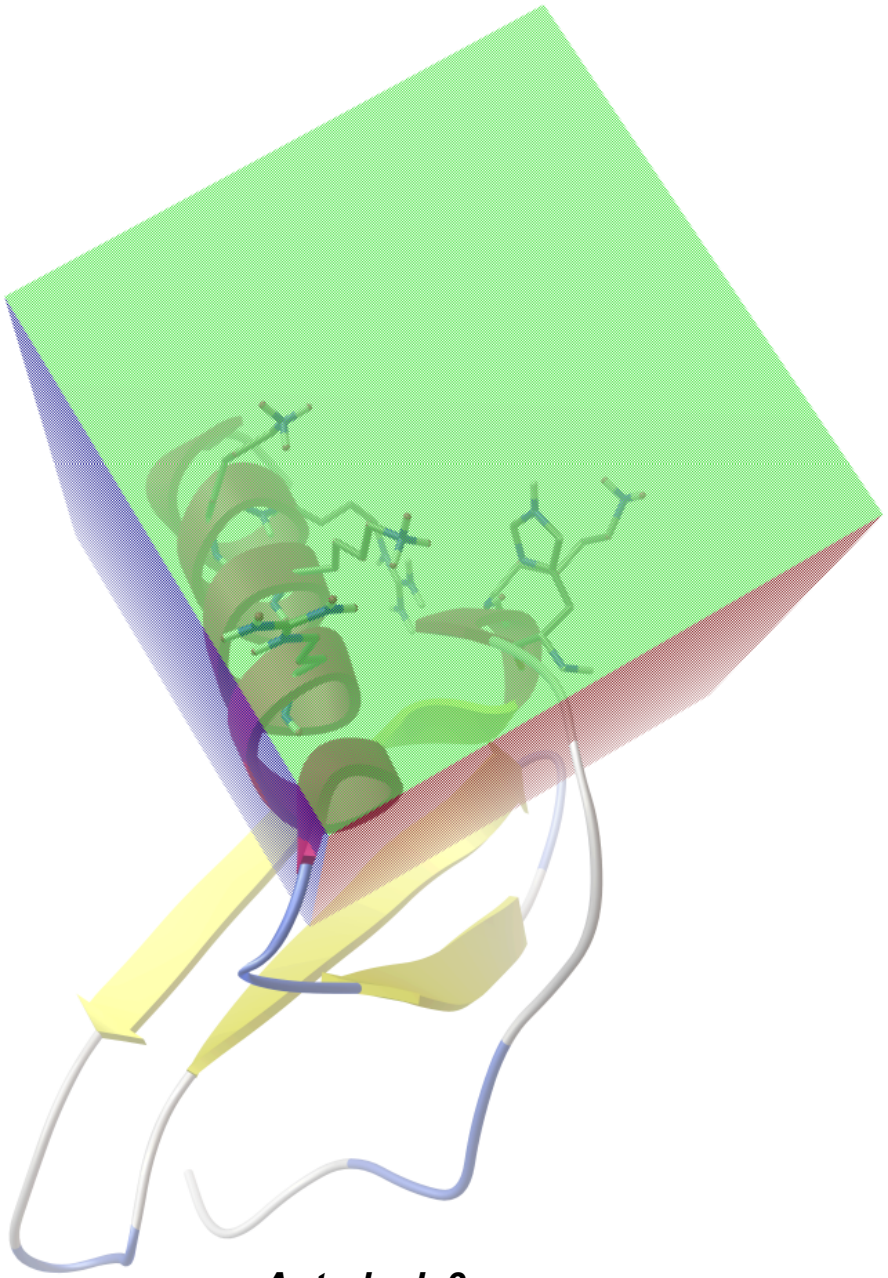
GOAL

to study GAGs recognition properties of IL-8 analyzing its interactions with HA, CS and their sulfated derivatives complementing MD and NMR studies

OUTLINE

- Docking GAGs to monomeric IL-8
- Binding pose energy analysis
- Complementation of MD and NMR results
- Specificity of GAGs binding vs electrostatics
- Analysis of bound GAGs elongation
- Docking GAGs to dimeric IL-8
- GAGs binding vs IL-8 dimerization

DOCKING GAGs TO IL-8: INPUT

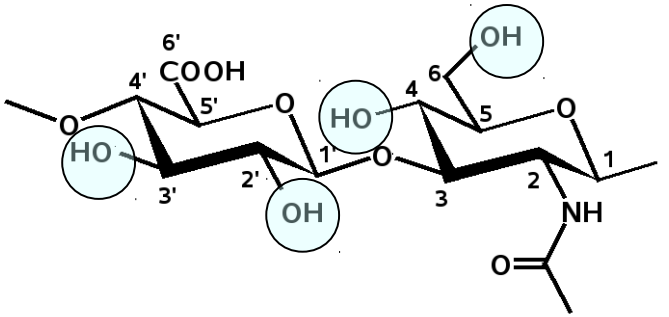


Autodock 3

- 3IL8 (2.00 Å), monomer (10-77)
- Box around heparin binding site
- Ligands: 14 flexible tetra-GAGs

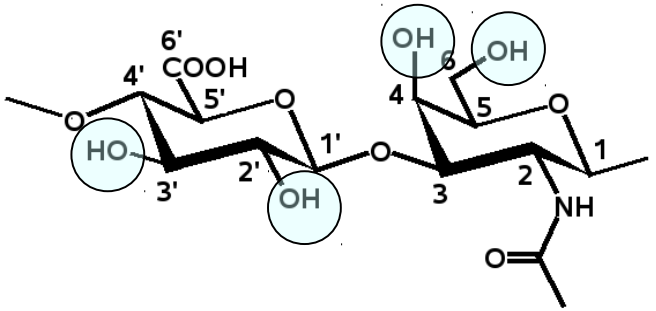
Hyaluronic acid (PDB ID: 2BVK):

HA, HA4, HA6, HA46, HA462', HA463', HA462'3'

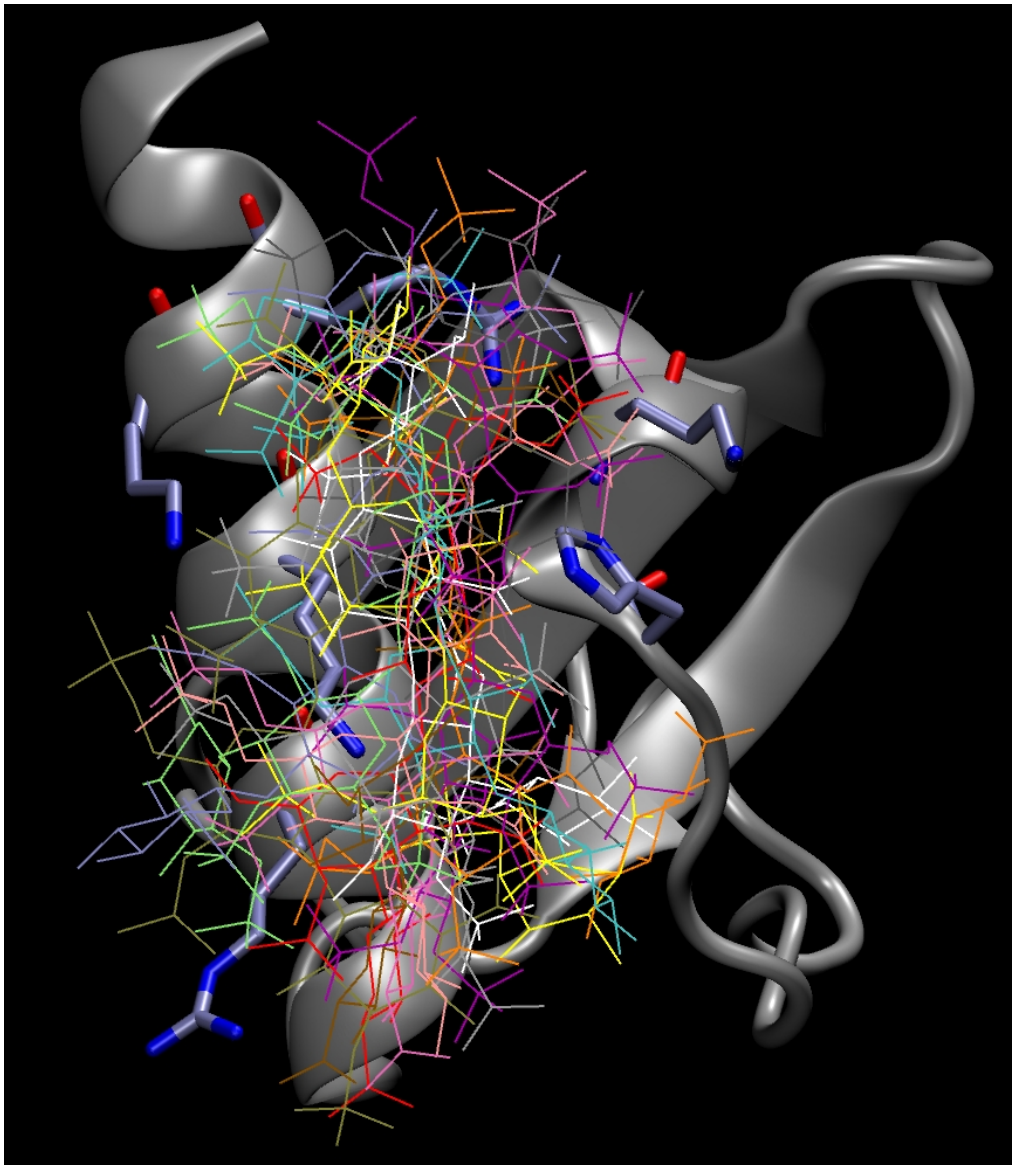


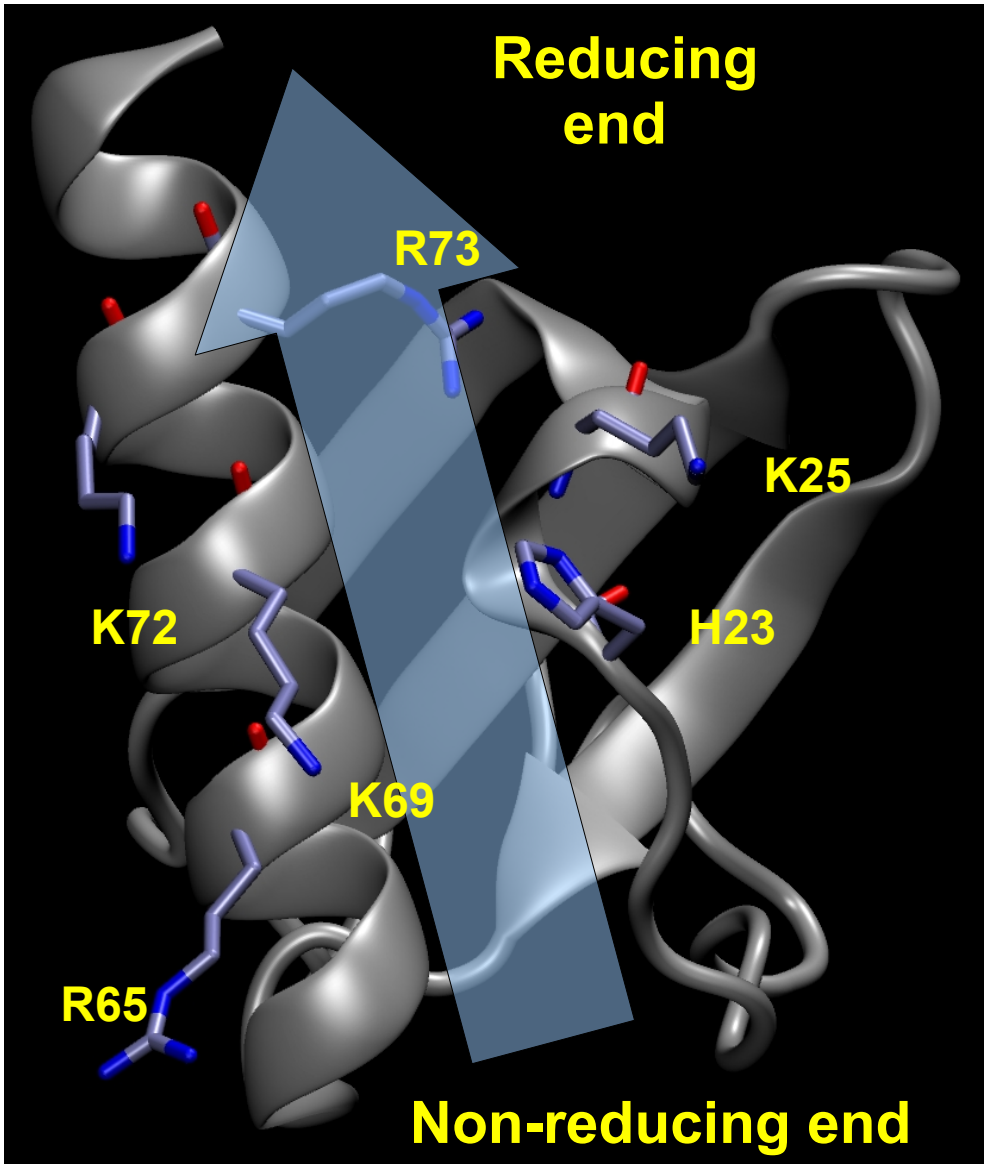
Chondroitin sulfate (PDB ID: 1C4S):

CS, CS4,CS6, CS46, CS462', CS463', CS462'3'



DOCKING OF GAGs TO IL-8: RESULTS

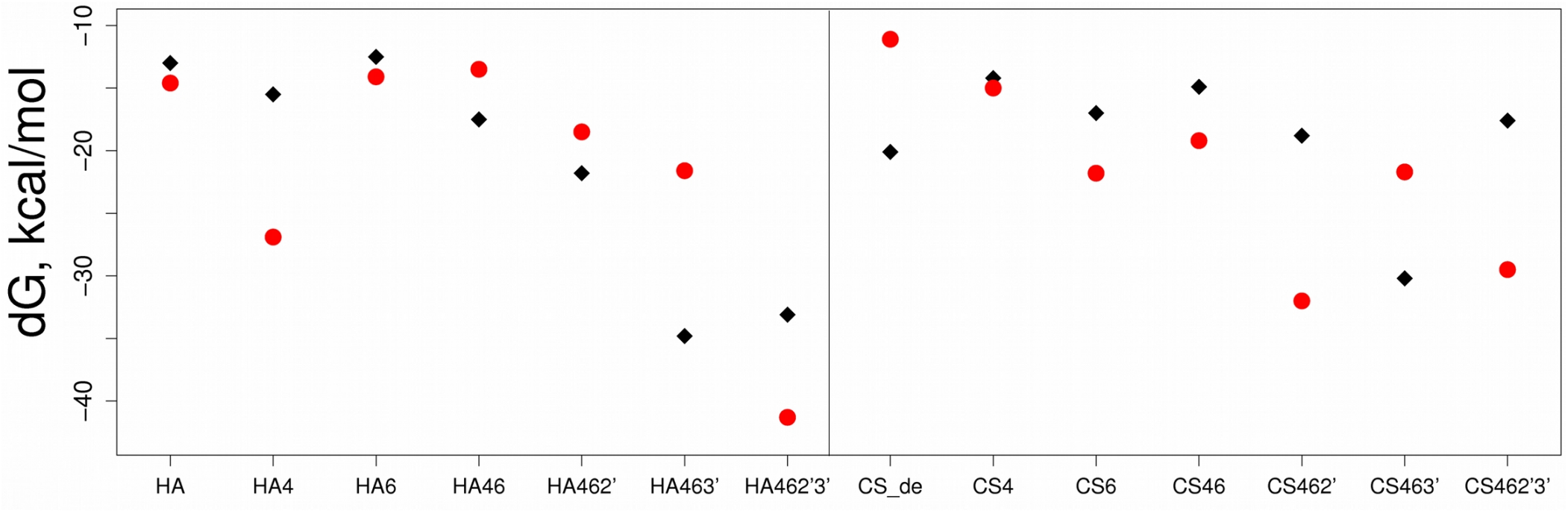




Highly scored and well represented pose for different GAGs

BINDING POSE ENERGY ANALYSIS

MD: 10 ns, AMBER99 and GLYCAM06 ff, PBC, counter ions, MM-PBSA

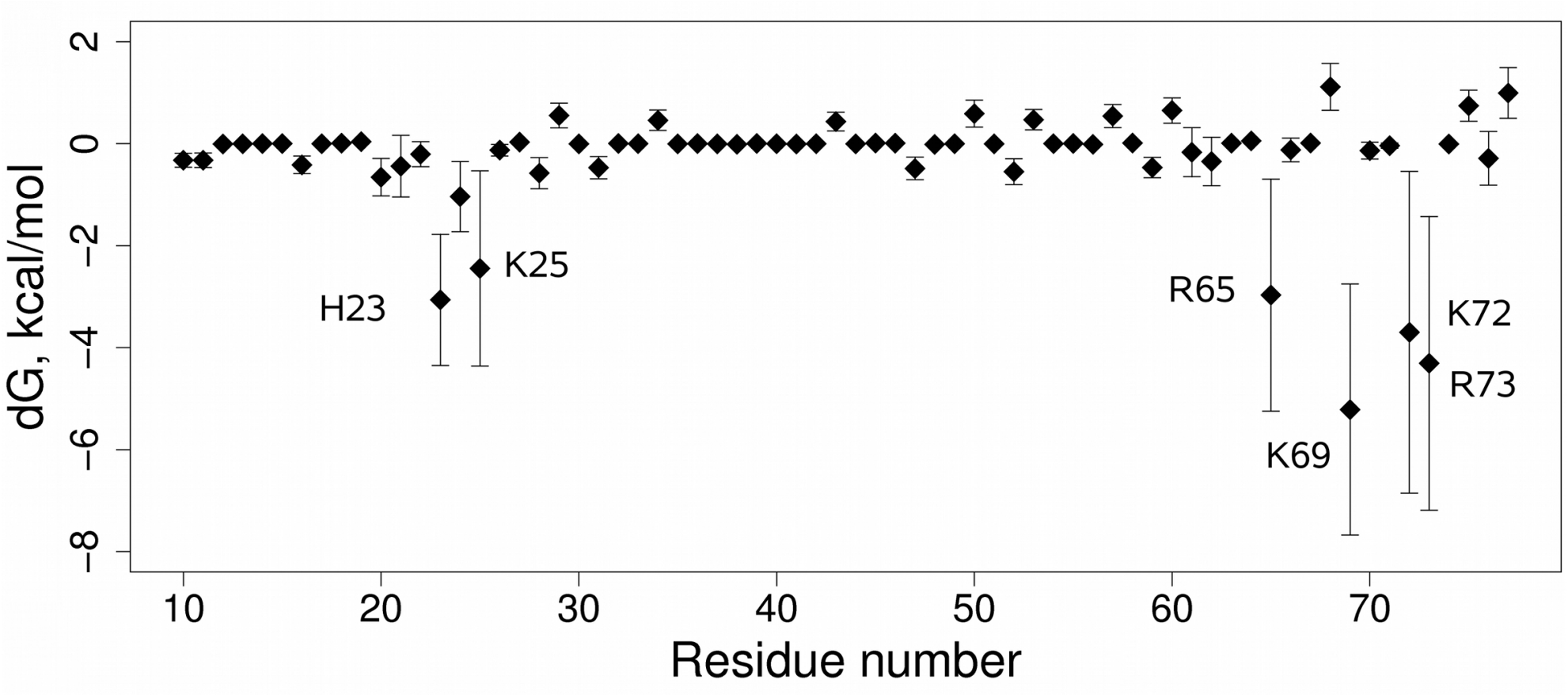


HA, sulfation increase

CS, sulfation increase

Increase of HA and CS sulfation favours binding to IL-8

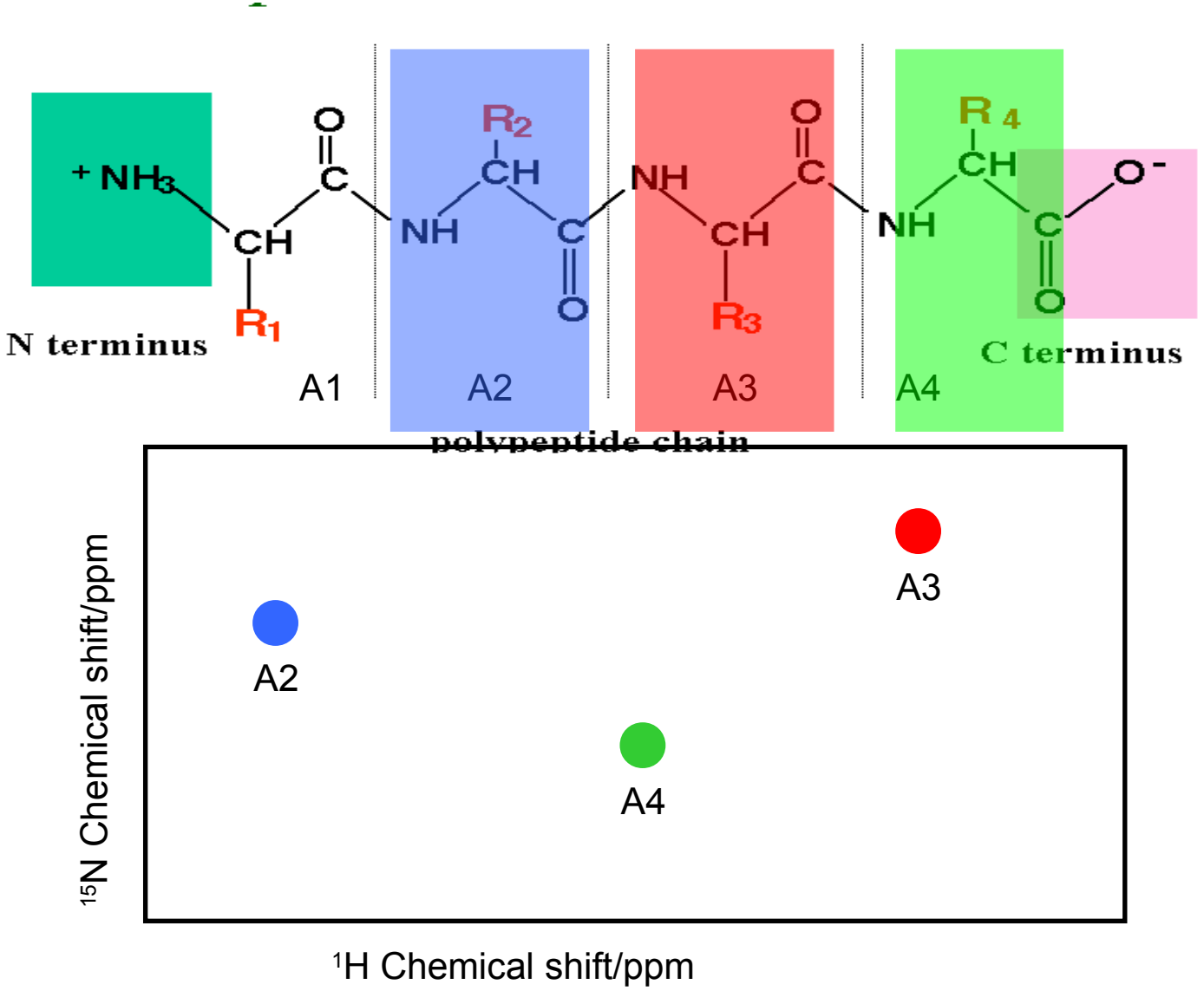
PER RESIDUE ENERGY DECOMPOSITION



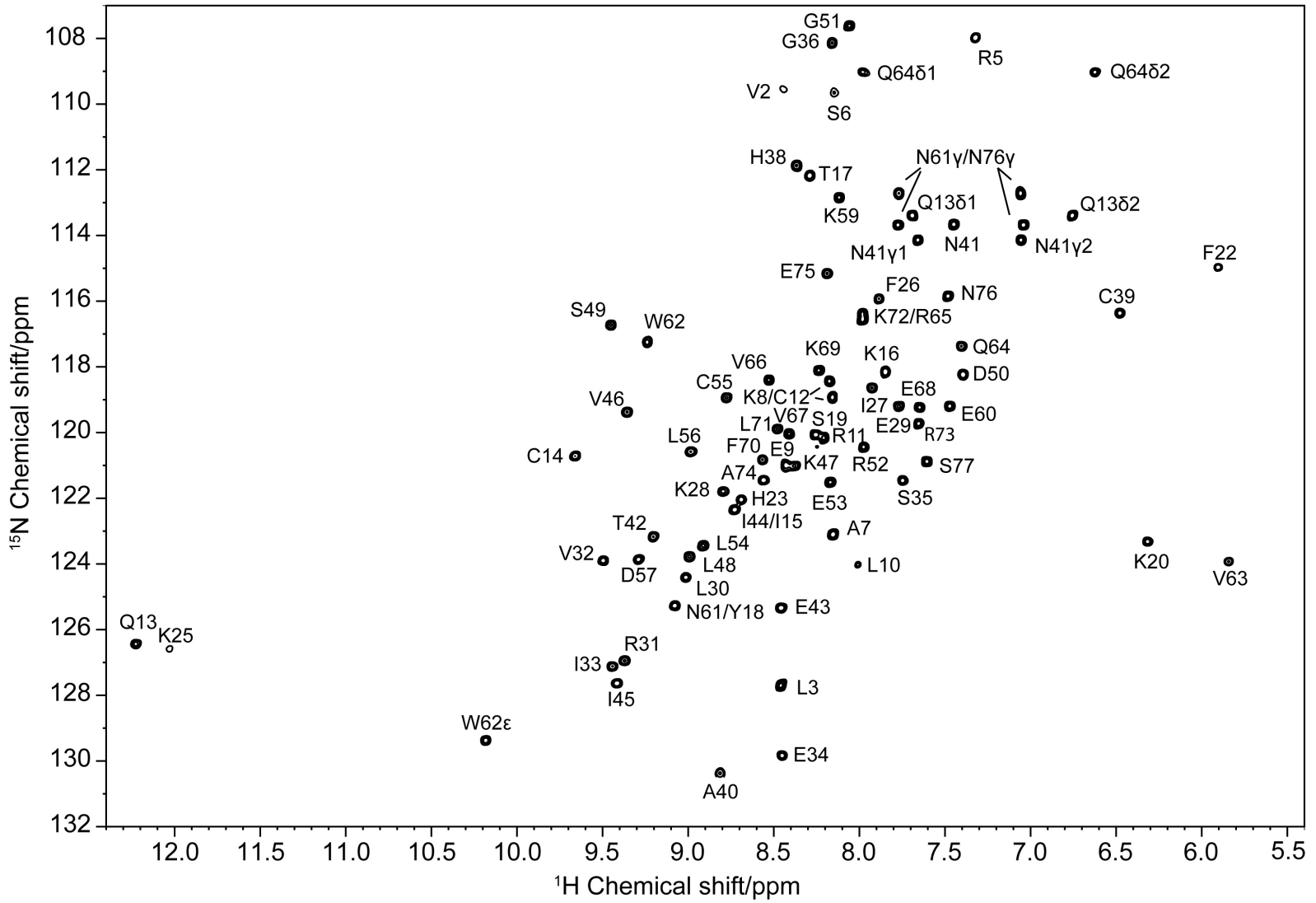
Pose energetic profile agrees with experimental data from mutagenesis

NMR: HSQC SPECTRUM

- ^{1}H-^{15}N HSQC spectrum: Heteronuclear Single-Quantum Coherence



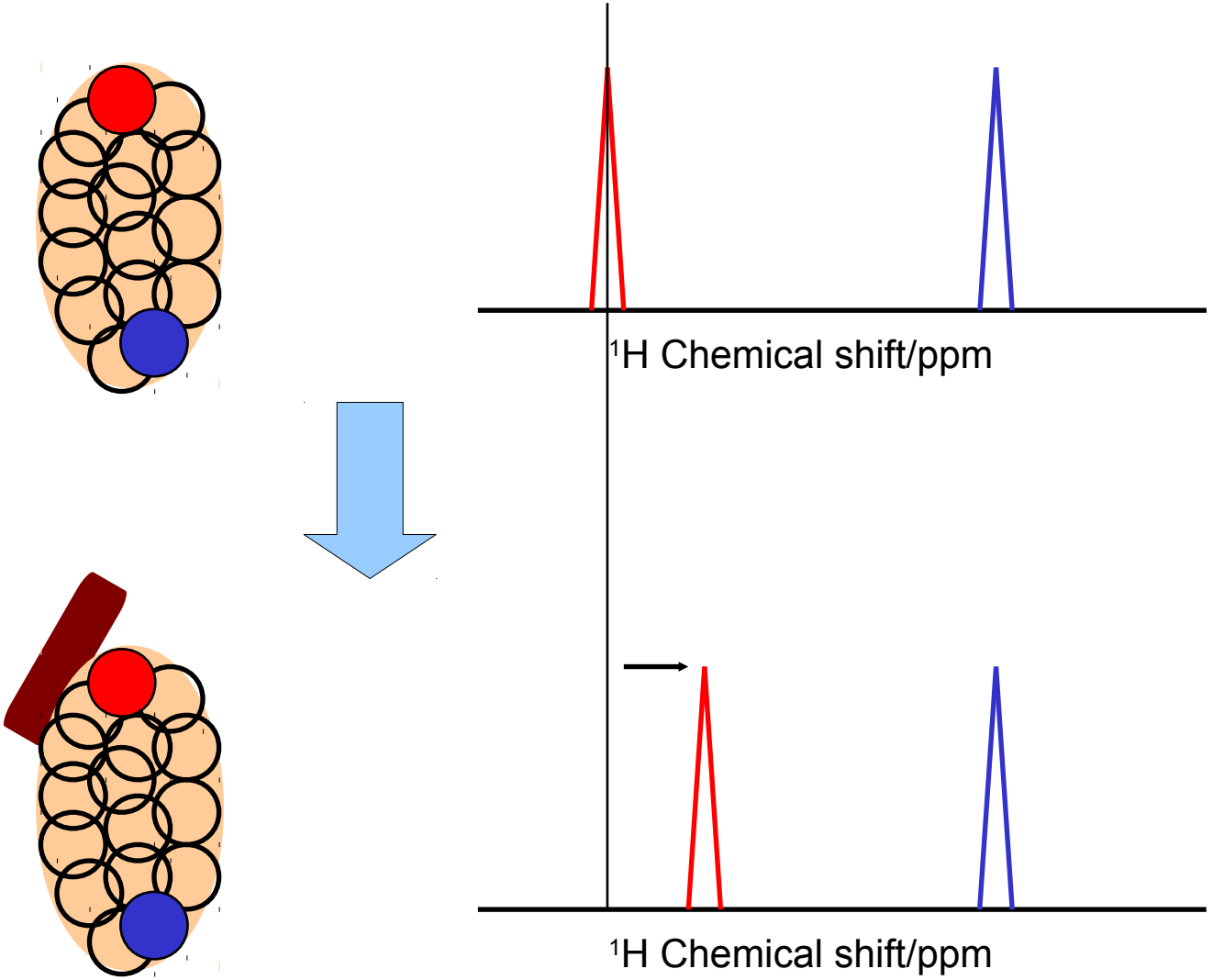
NMR: IL-8 AMINO ACIDS ASSIGNMENT



^{1}H-^{15}N HSQC spectrum (Heteronuclear Single-Quantum Coherence)

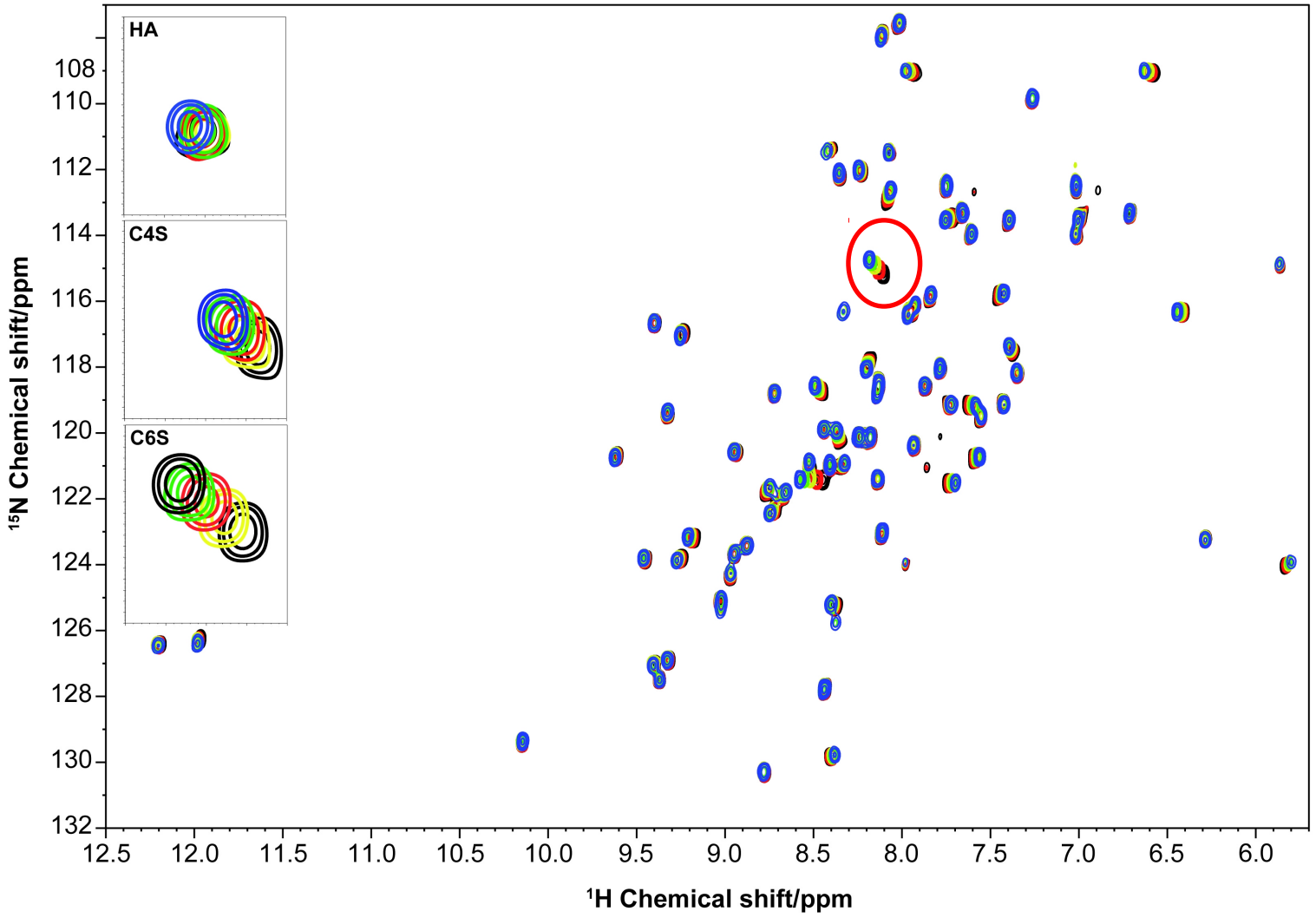
77 amino acids

NMR TITRATION: PRINCIPLE



$$\Delta\delta = \sqrt{\left(\Delta\delta H\right)^2 + \left(0.2\Delta\delta \mathrm{N}\right)^2}$$

IL-8 TITRATION STUDIES WITH GAGS



c(IL-8): 0.99 mM

c(C6S):

0.124 mM

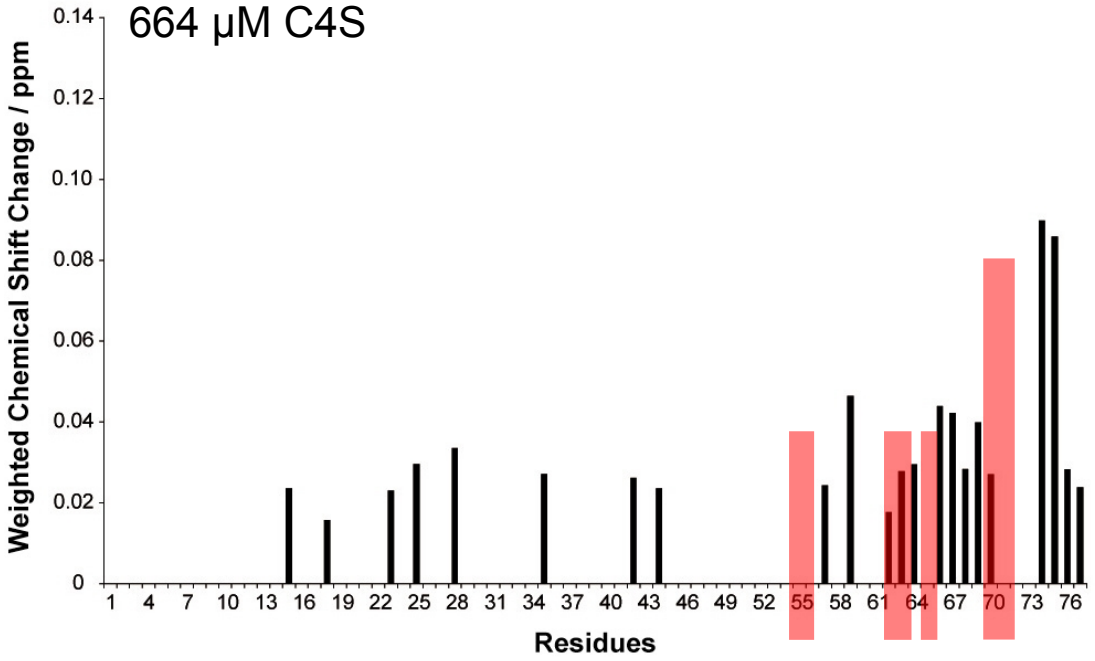
0.244 mM

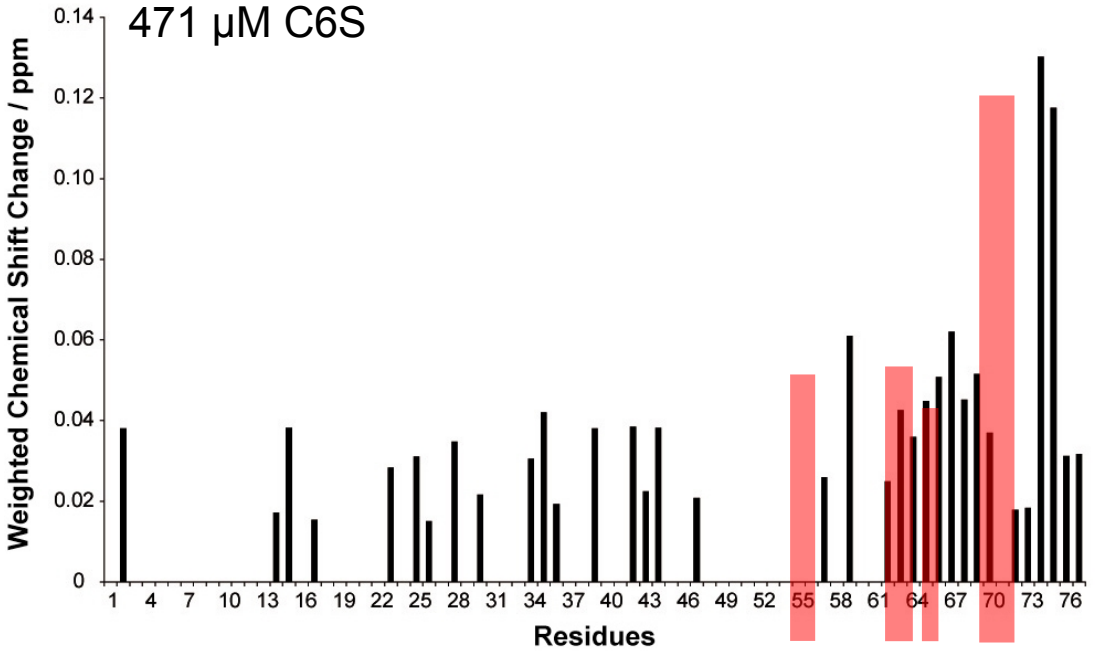
0.359 mM

0.471 mM

Chondroitin-6-sulfate hexasaccharide (C6S)

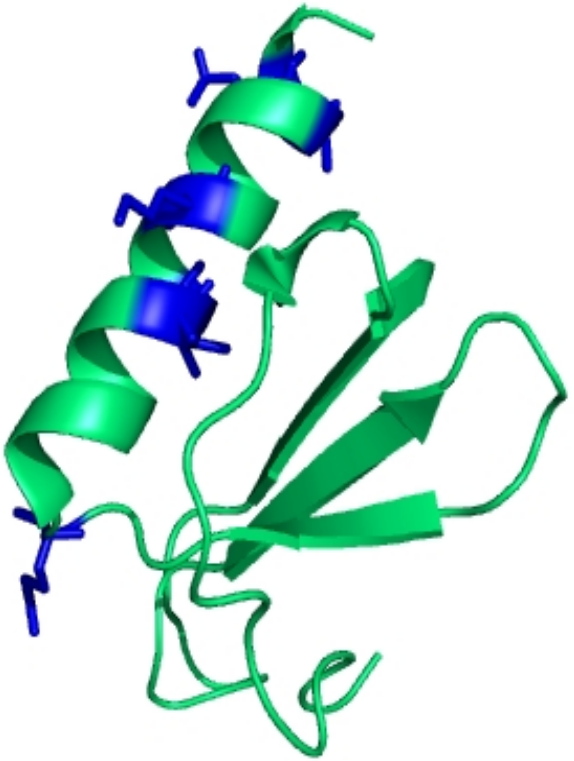
IL-8 TITRATION STUDIES WITH CS4 AND CS6



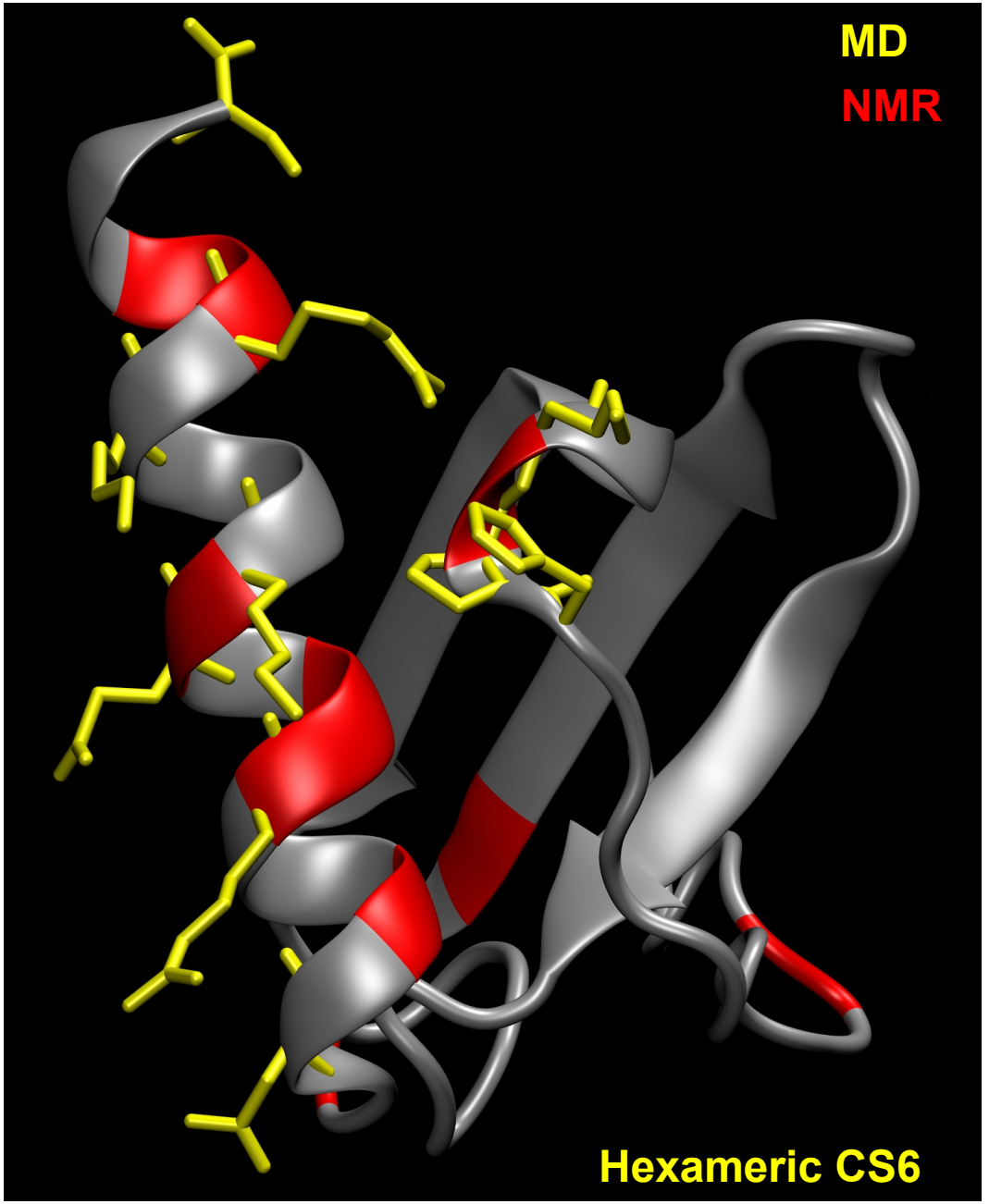


Largest chemical shift changes :

K59, V66, V67, K69, A74, E75

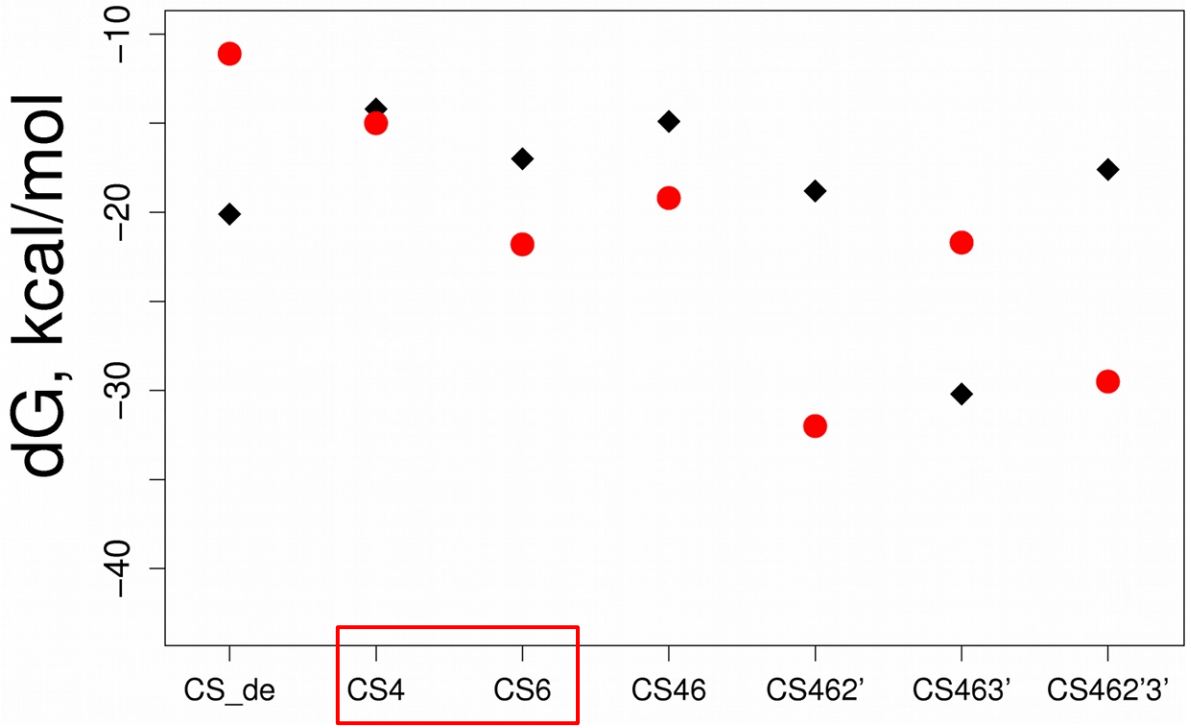


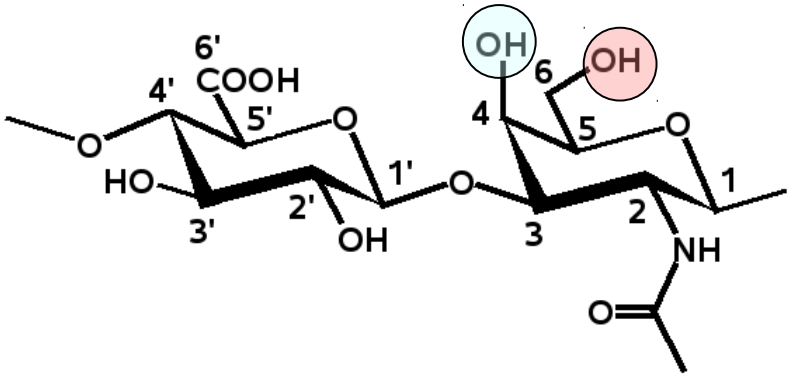
MD *VS* NMR



MD energies and NMR chemical shifts changes agree/complement

MD + NMR: DETECTING SPECIFICITY





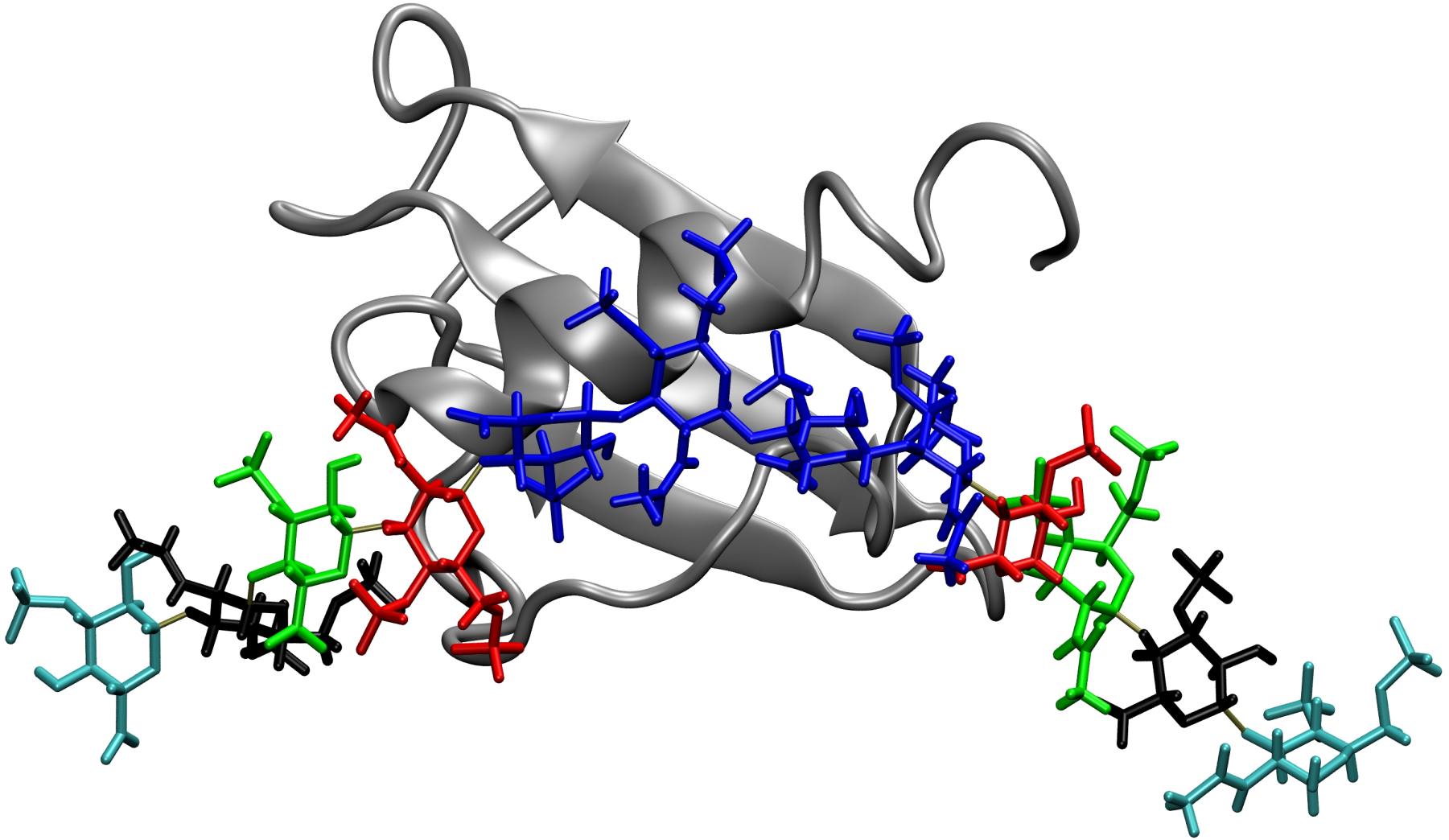
CS4 vs CS6

Difference: -3.6±4.2 kcal/mol

(4 MD tetra GAG; 2 MD hexa GAG)

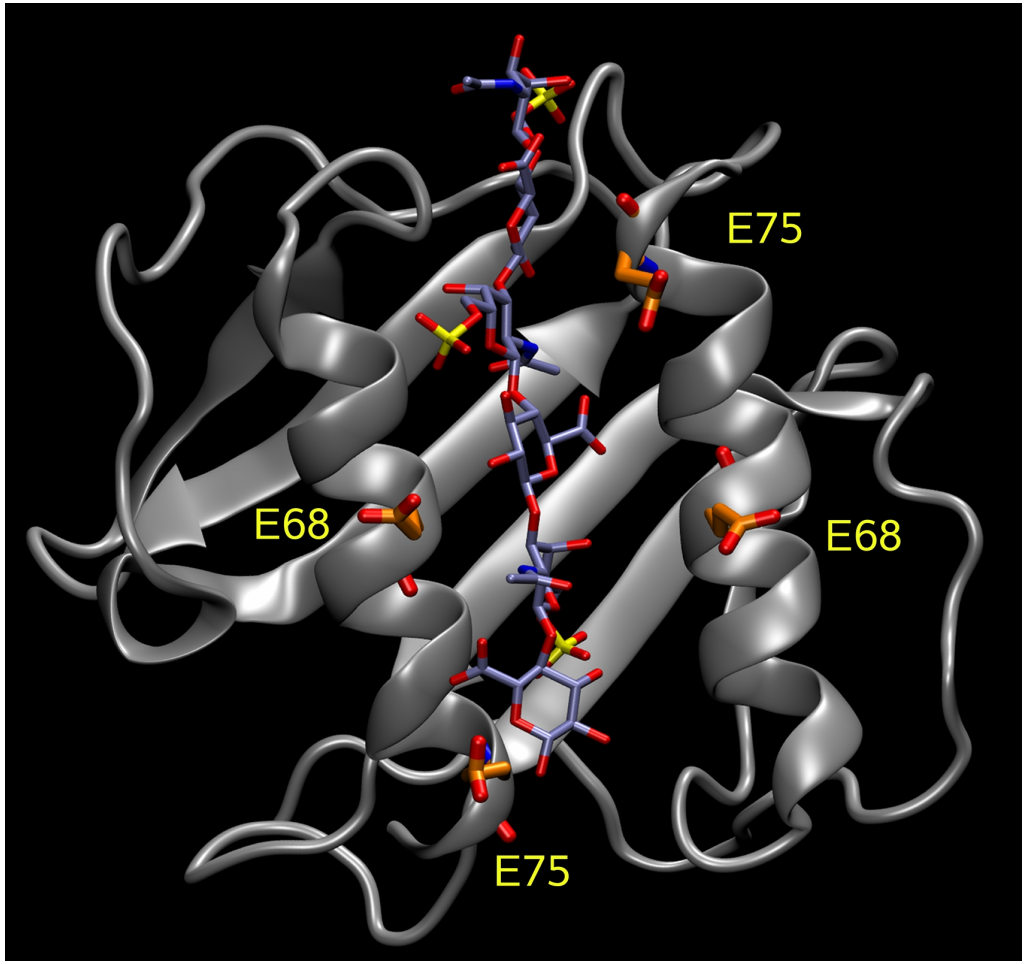
MM-PBSA and NMR find similar differences for CS4 and CS6 binding

ELONGATION OF BOUND GAGS

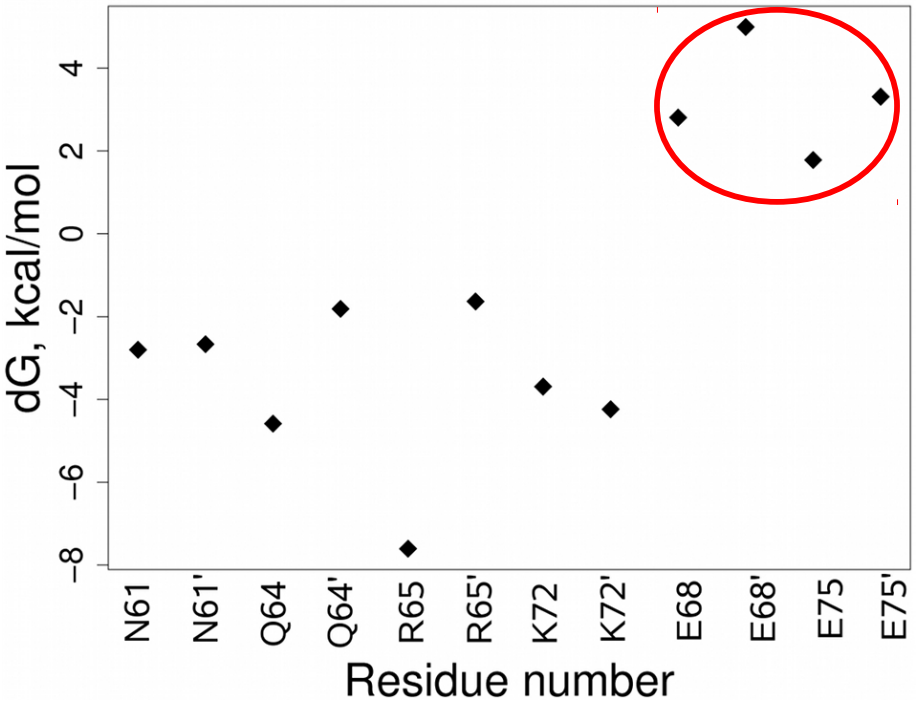


- Elongation of bound tetrameric GAG neither improves binding, nor changes the interaction pattern of IL-8
- Tetrameric GAG represents the essential specific unit for IL-8 binding

GAGs DOCKING TO DIMERIC IL-8



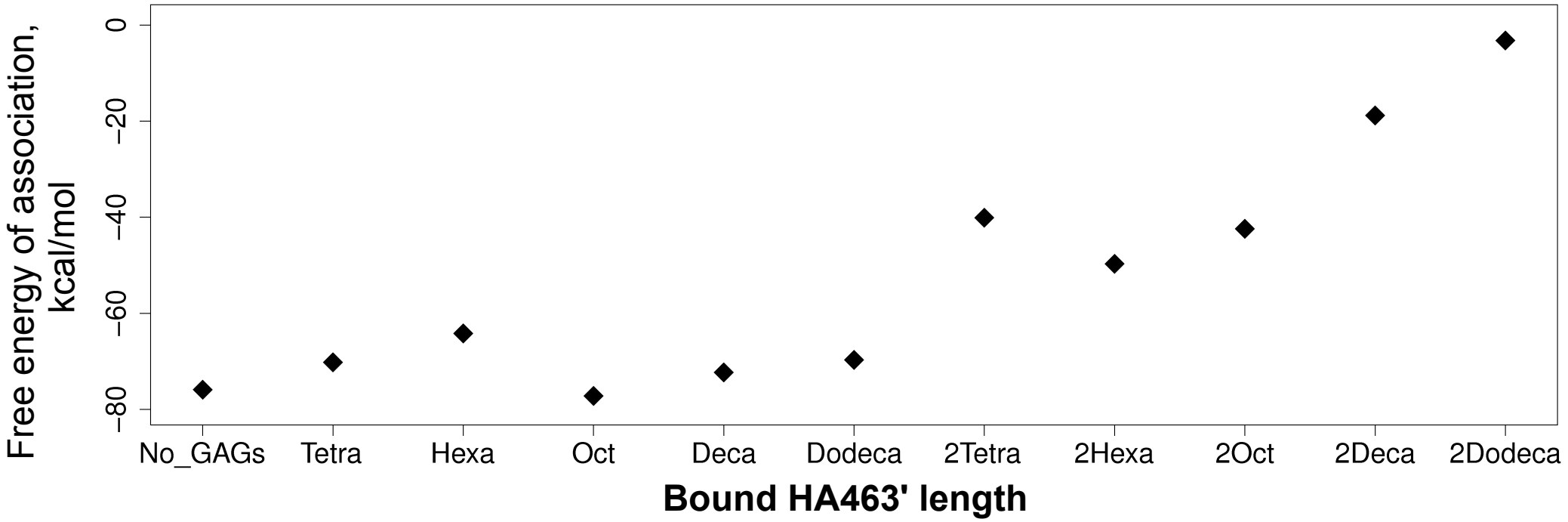
Dimeric IL-8 (PDB ID: 1IL8) + CS6

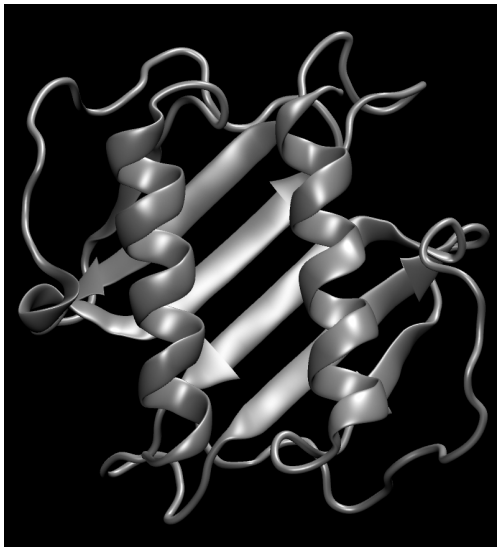


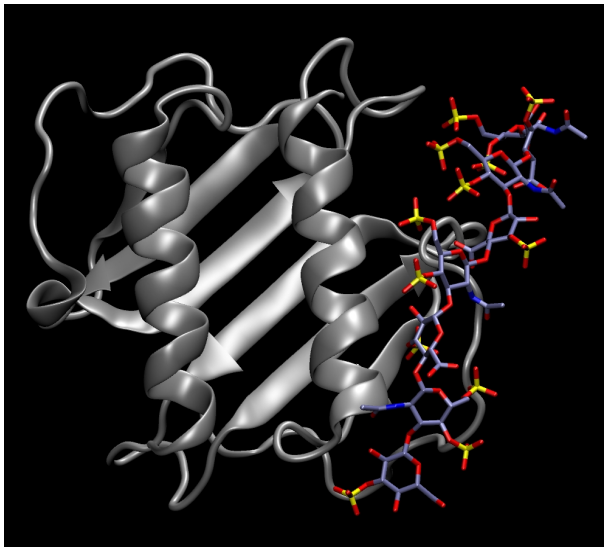
ΔG > 0 kcal/mol

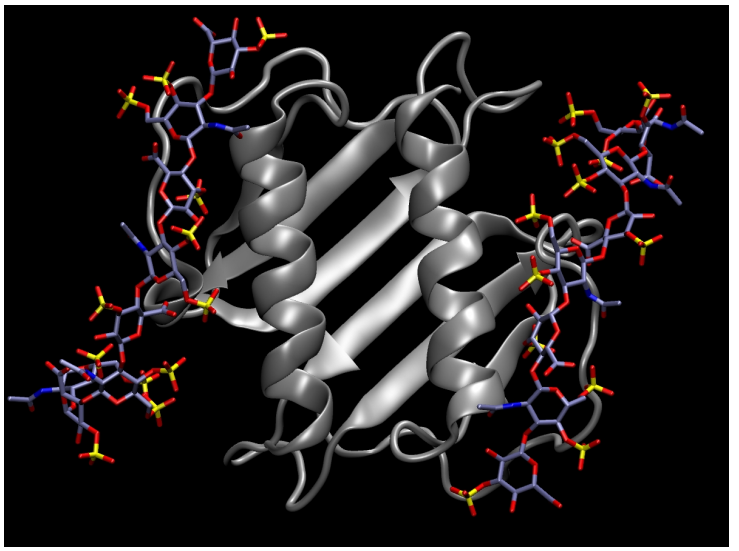
- **Alternative binding pose for dimeric IL-8 fails to demonstrate stability**
- **The same binding pose of GAGs for dimeric and monomeric IL-8**

GAGs BINDING VS IL-8 DIMERIZATION







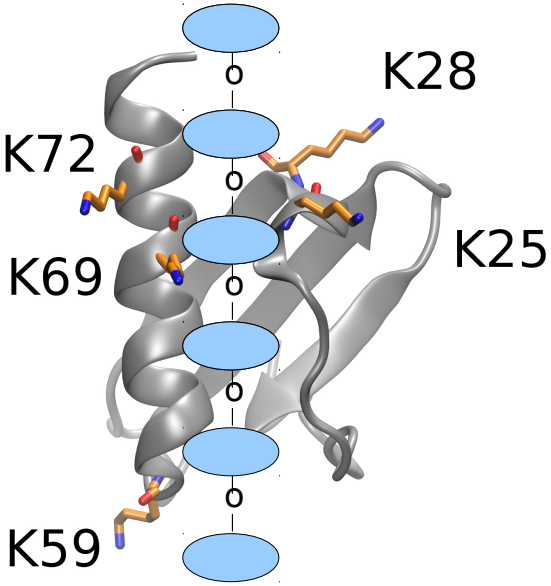


IL-8 monomers association is favoured by GAGs binding due to electrostatics

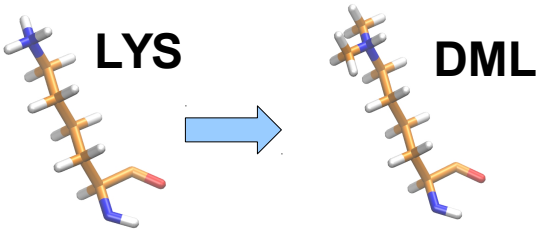
INDIVIDUAL IMPACT OF LYS RESIDUES

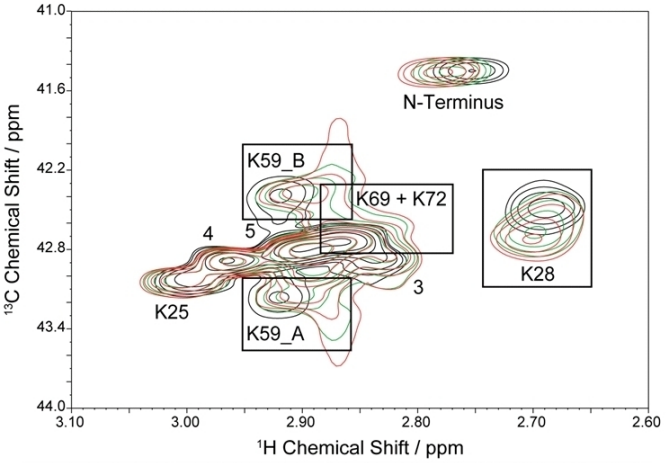
Receptor IL-8: WT, all DML, DML[25,28,59,69,72]Q

Ligand: hexa HA, CS4, CS6, DS, HE



- Dimethylation effect depends on GAGs
- K59 ≤ K28 << K25 < K69 ≈ K72

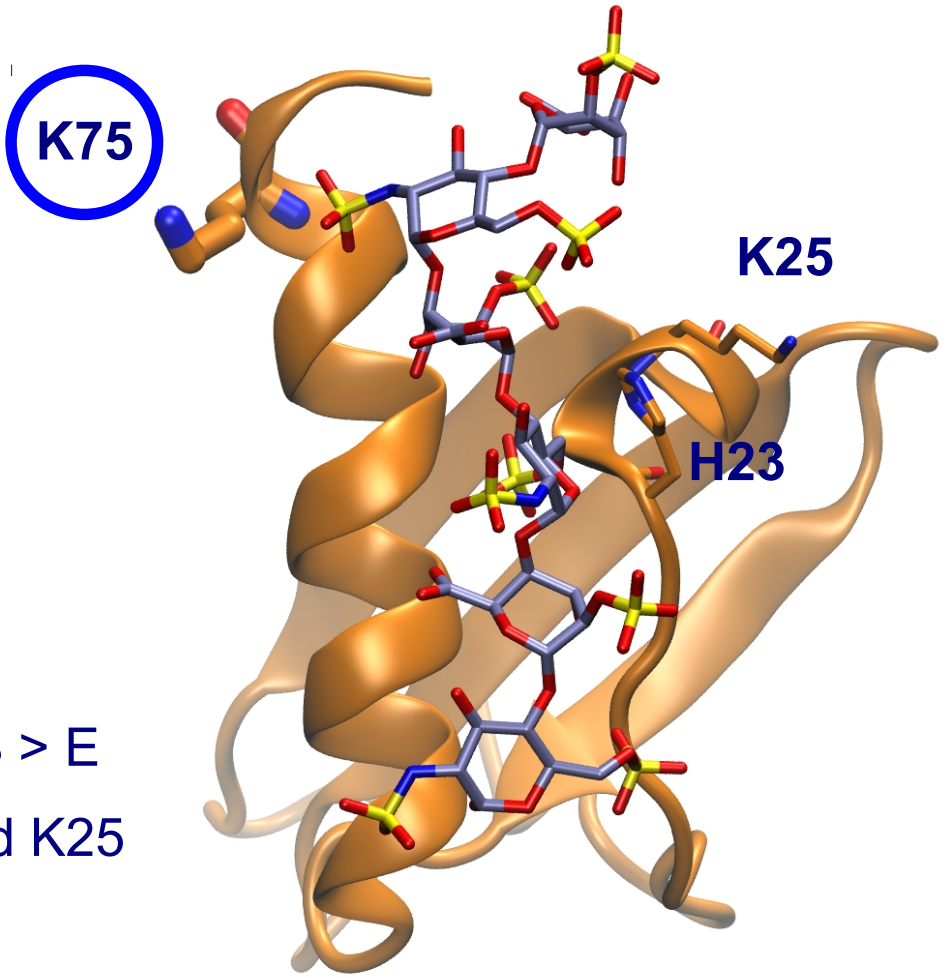




THE ROLE OF THE RESIDUE IN POSITION 75

Receptor IL-8: WT, E75K

Ligand: hexa HA, CS6, HE

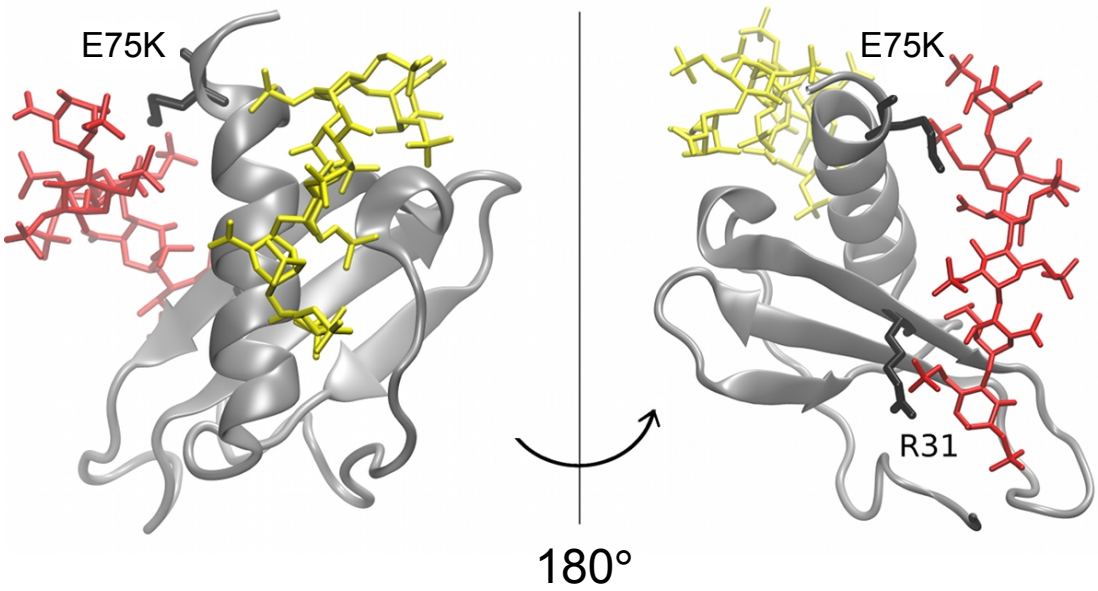


- Binding GAGs: E75K > WT
- Other *in silico* mutations 75: K,R > A,S > E
- Increased energetic impact of H23 and K25

THE ROLE OF THE RESIDUE IN POSITION 75

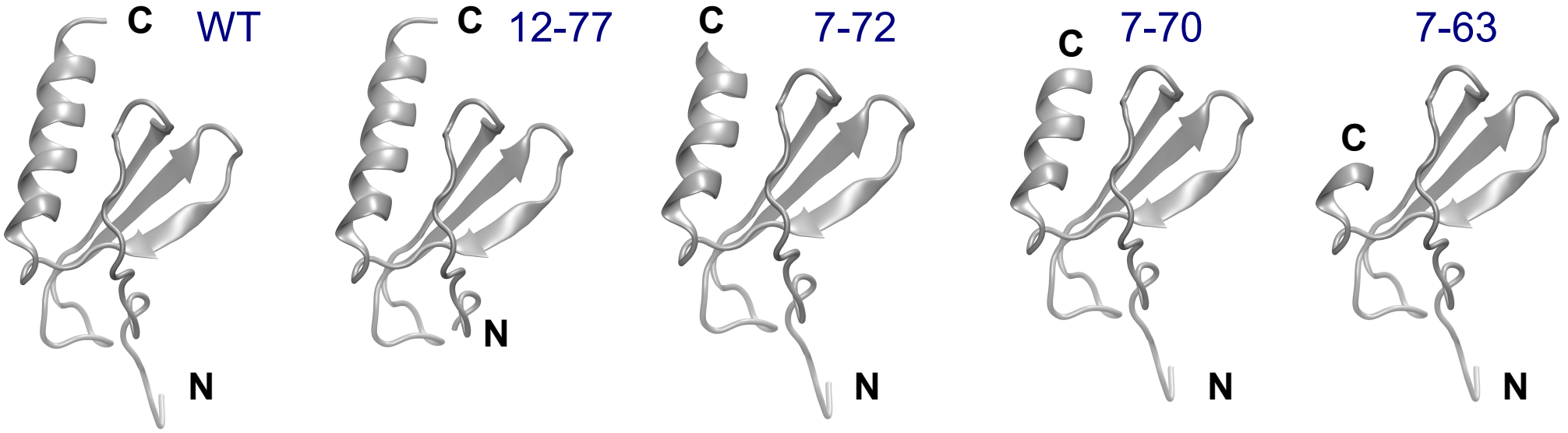
Receptor IL-8: WT, E75K

Ligand: hexa HA, CS6, HE



- Binding GAGs: E75K > WT
- Other *in silico* mutations 75: K,R > A,S > E
- Increased energetic impact of H23 and K25
- Additional binding pose only for the mutant (through R31)

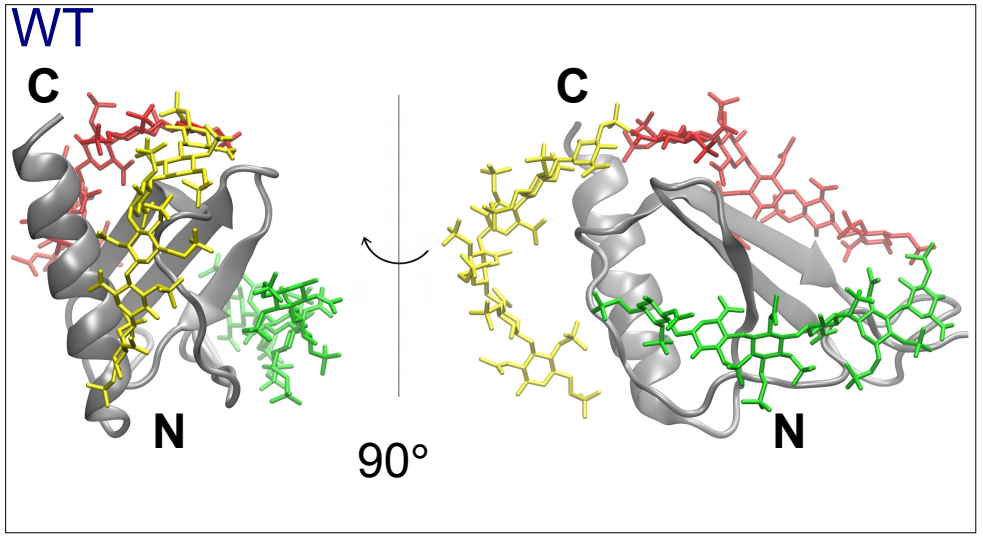
THE ROLE OF N- AND C-TERMINI: TRUNCATED MUTANTS

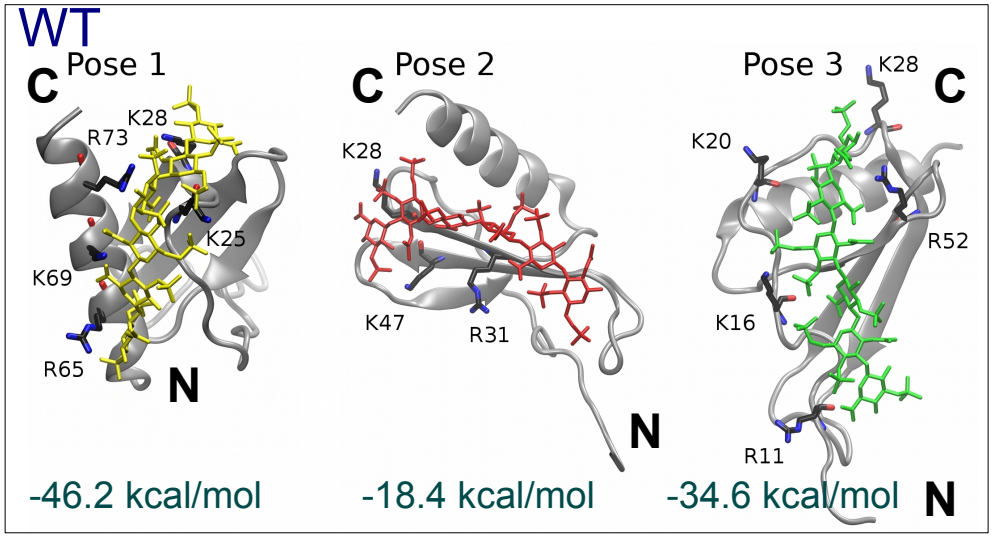


Receptor IL-8: WT, 12-77, 7-72, 7-70, 7-63

Ligand: hexa HA, HA6, HA462', HA463', HE

- N-terminal truncation: no effect
- C-terminal truncation: strong effect on GAG binding strength and pose





CASE STUDY: SUMMARY

- Structures of IL-8 complexes with GAGs?
  - We find highly scored and representative GAGs binding pose
- Quantitative impacts of individual IL-8 residues?
  - We find the residues crucial for GAGs binding
- Specific binding for different GAGs or purely electrostatics?
  - Increase of sulfation improves binding though specificity is also observed
- The size of essential GAG unit for IL-8 specific binding?
  - Tetrameric GAG is essential minimal unit
- GAGs influence on IL-8 dimerization?
  - Binding two GAGs to dimeric IL-8 assists dissociation of the dimer

LECTURE 7: QM, MD AND NMR

- Basics of NMR
- NMR and QM: GIAO method
- NMR and MD: Karplus equation
- Software for calculation NMR parameters
- Case study 1: GIAO calculations for saccharides
- Case study 2: IL-8 interactions with GAGs by NMR and MD