Using NMR to study Macromolecular Interactions

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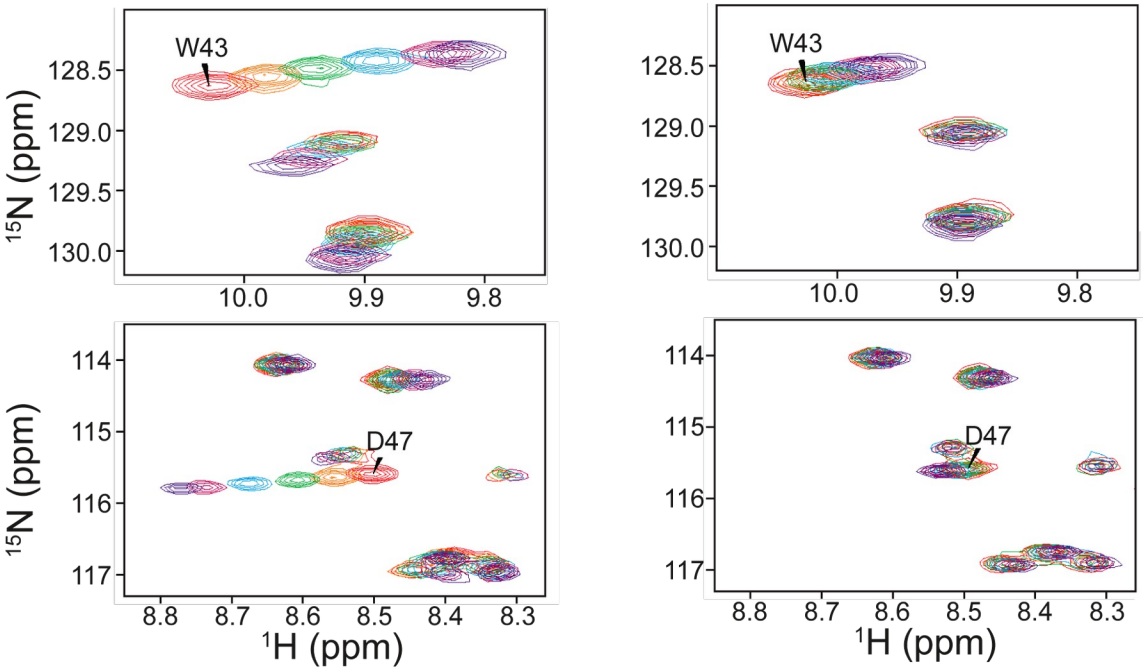
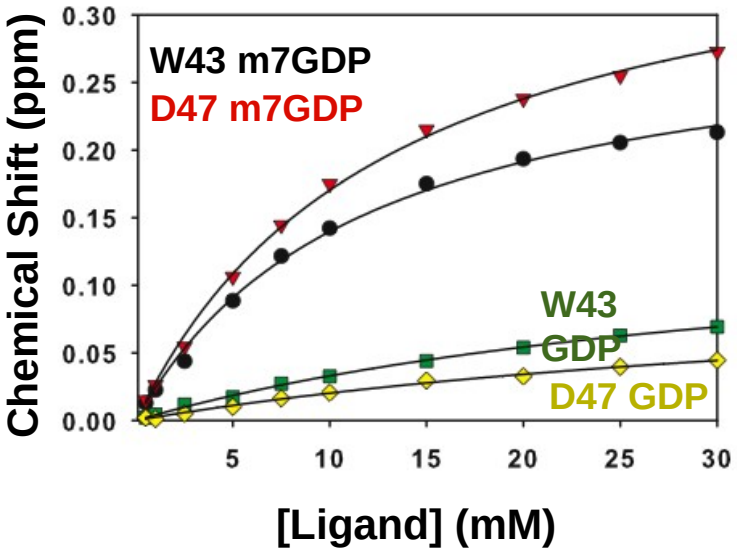
Outline

- Multidimensional NMR
- Macromolecular Interactions
- Dynamics
- Dealing with large complexes
- Structure Determination

Part II:
Macromolecular Interactions Detected by NMR

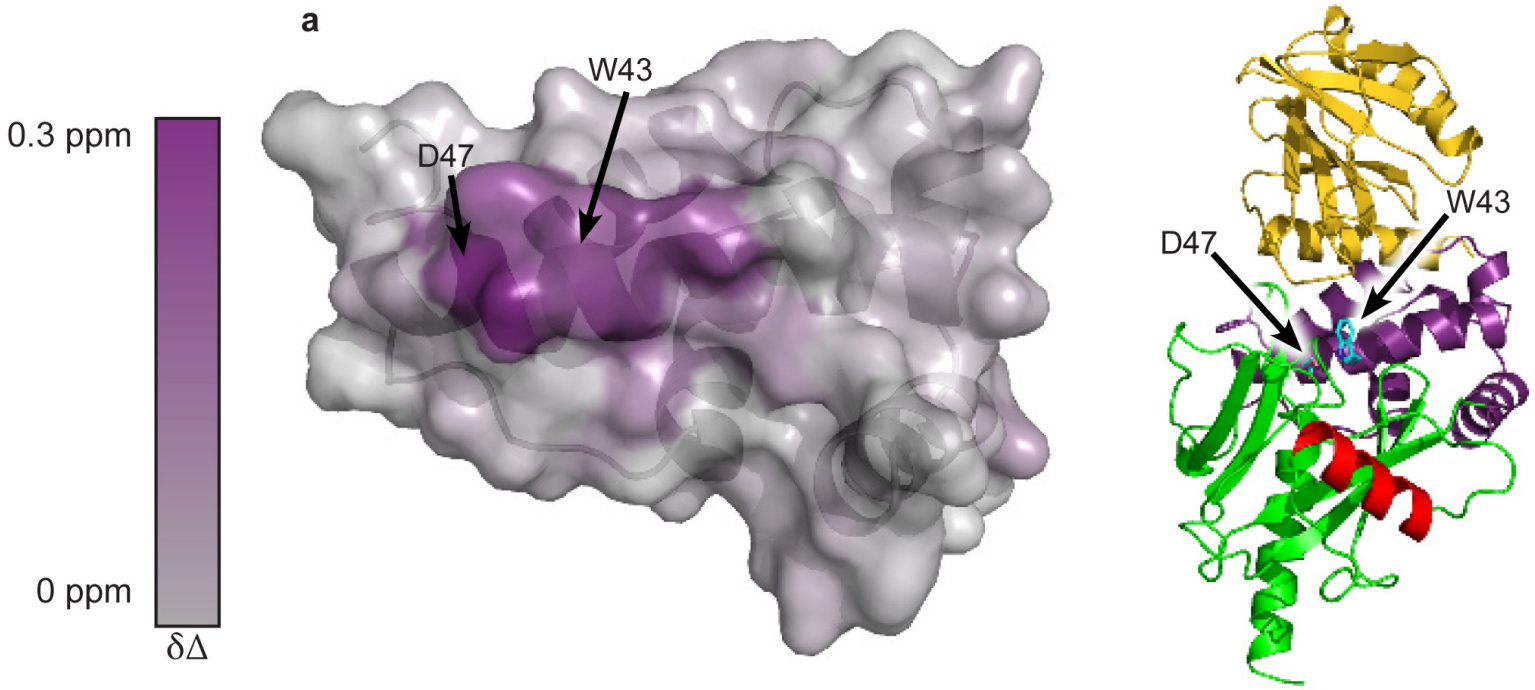
Binding of nucleotide to protein





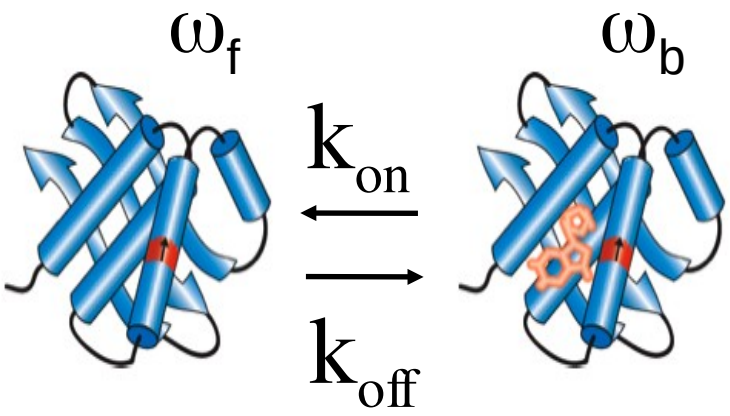
Dose dependent resonance shifts can be fit to obtain Kd

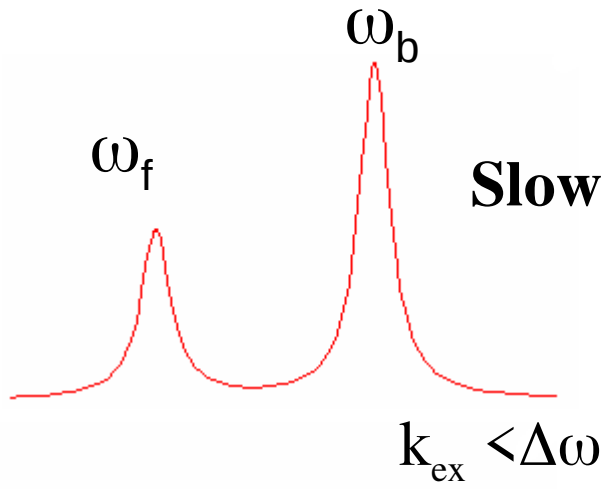
Shifts may be color coded onto surface to identify ligand binding site



Caveats?

NMR to monitor ligand binding

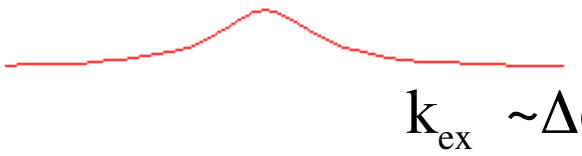


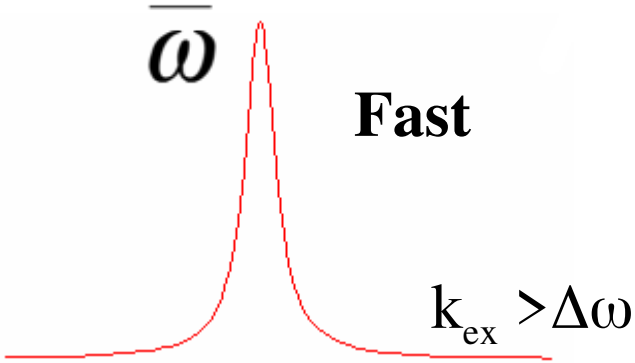


$$k_{ex} = k_{on}[L] + k_{off}$$

$$\Delta\omega = \omega_f - \omega_b$$

Intermediate



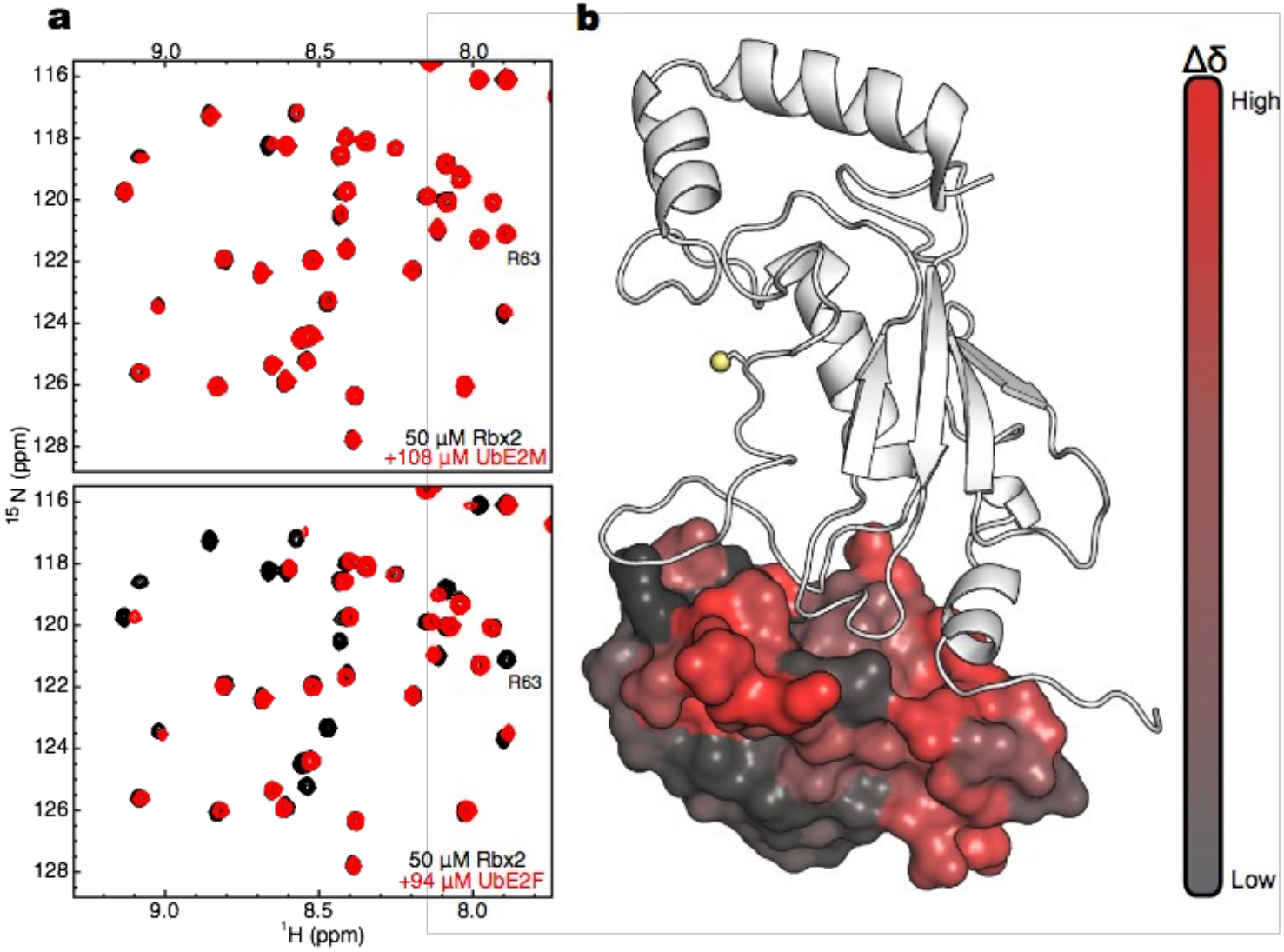


Fraction bound of labeled protein

$$P_b = \frac{\bar{\omega} - \omega_f}{\omega_b - \omega_f} = \frac{[L]}{[L] + K_d}$$

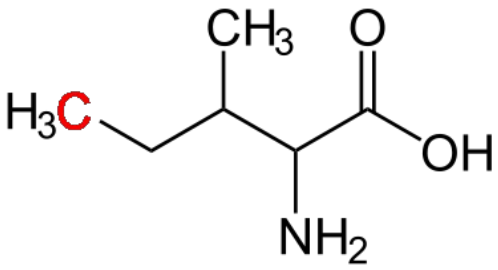
$\bar{\omega}$: observed chemical shift

Monitoring Protein/Protein Interactions by HSQC

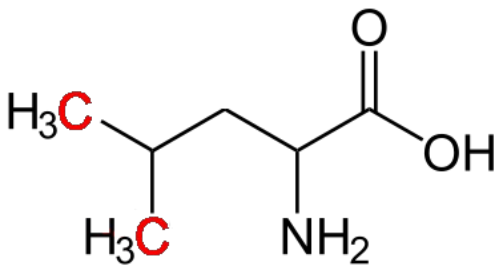


Sparse Labeling to Simplify Spectra

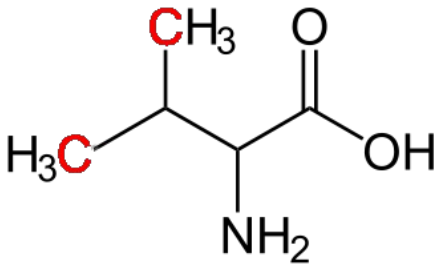
Selectively label R group methyls with C-13 (NMR visible)



Isoleucine



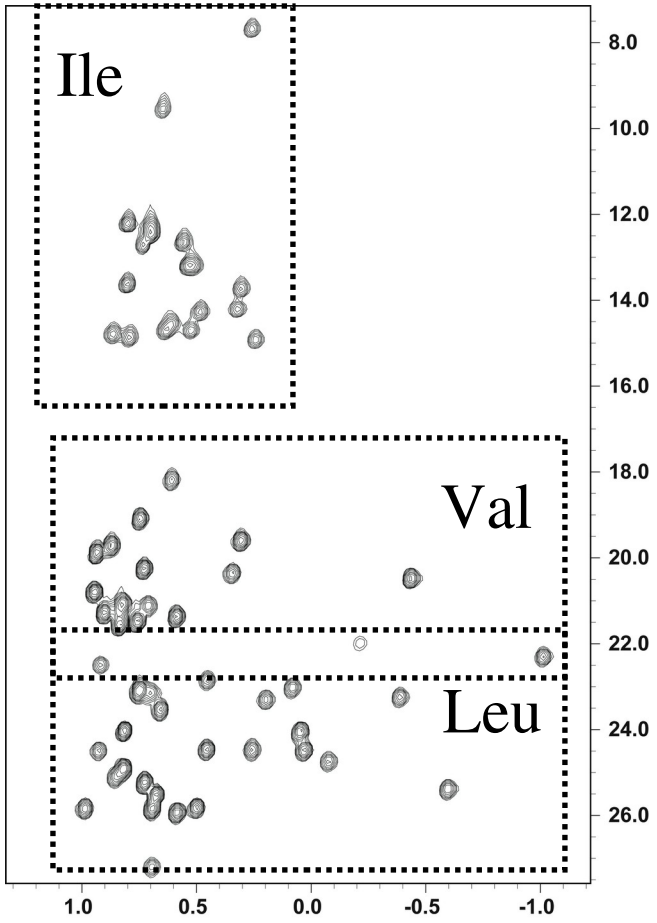
Leucine



Valine

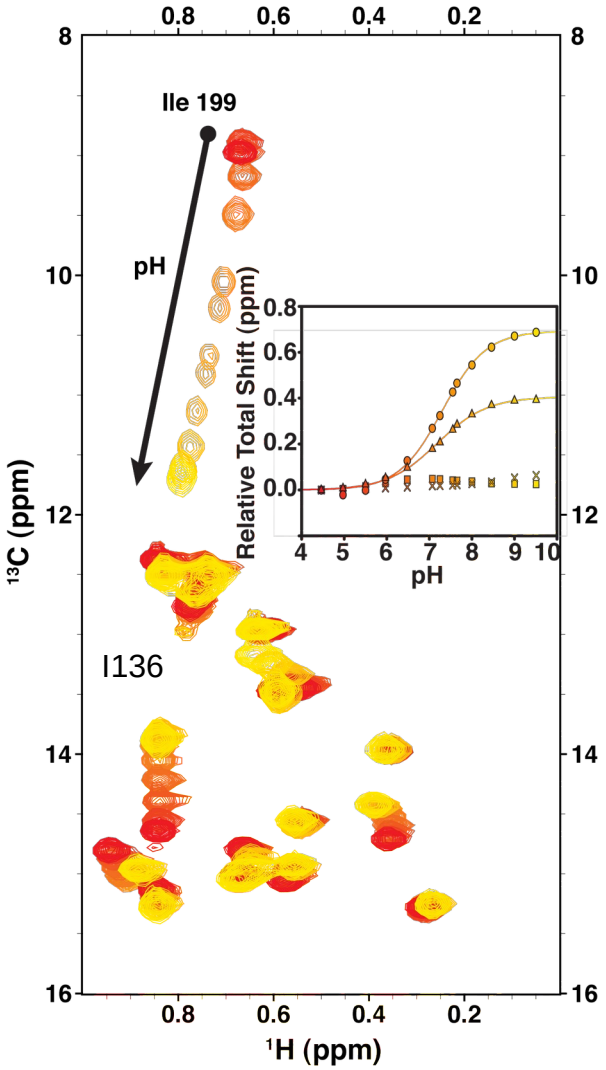
(add alpha-ketoacid precursors to ILV 30 minutes prior to induction)

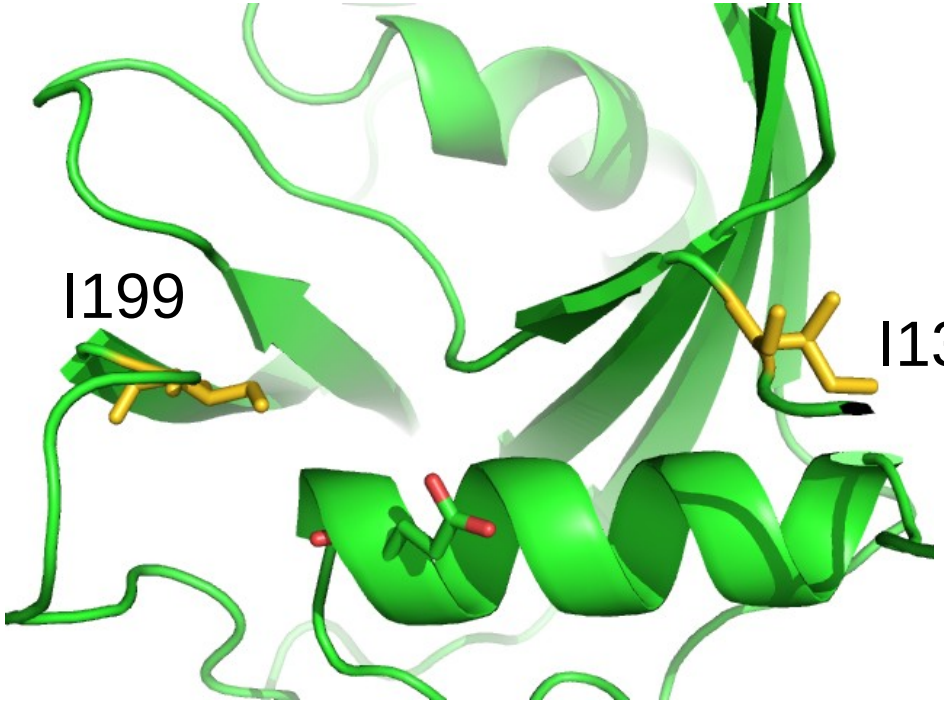
^{13}C HSQC of ILV labeled protein



Measuring pKa by NMR

(pH 4.5 - 9.5)





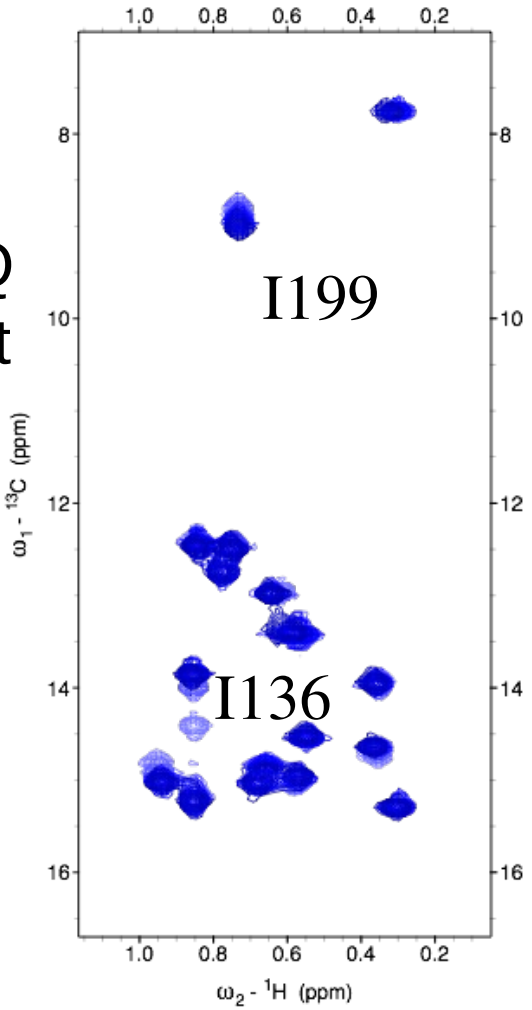
pKa of 7.2, elevated for Glu

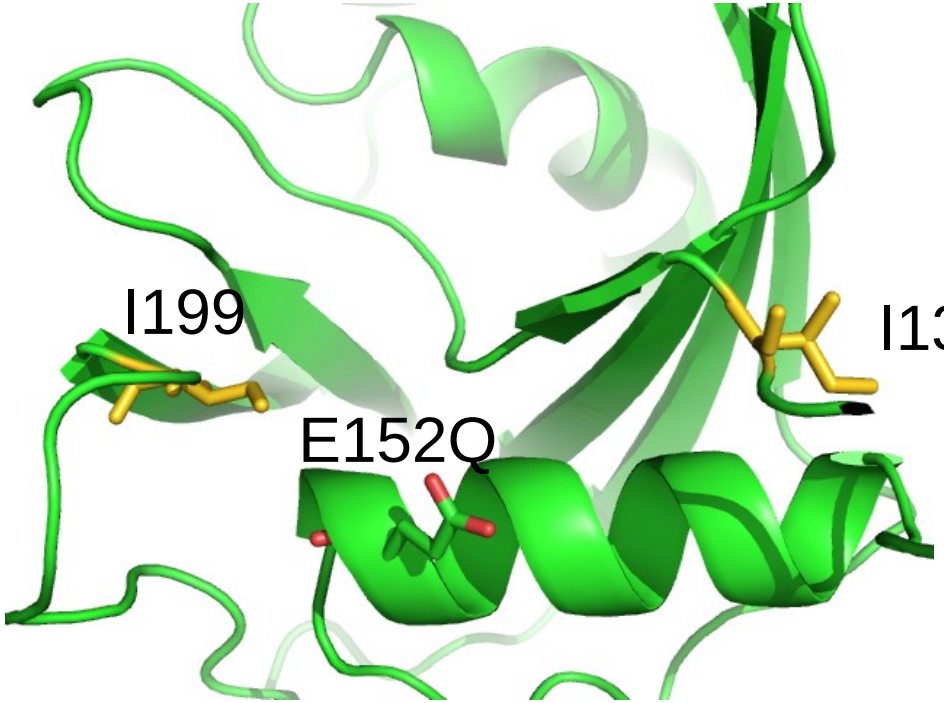
Aglietti et al, Structure 2013

Identification of titratable residue by site-directed mutagenesis and NMR

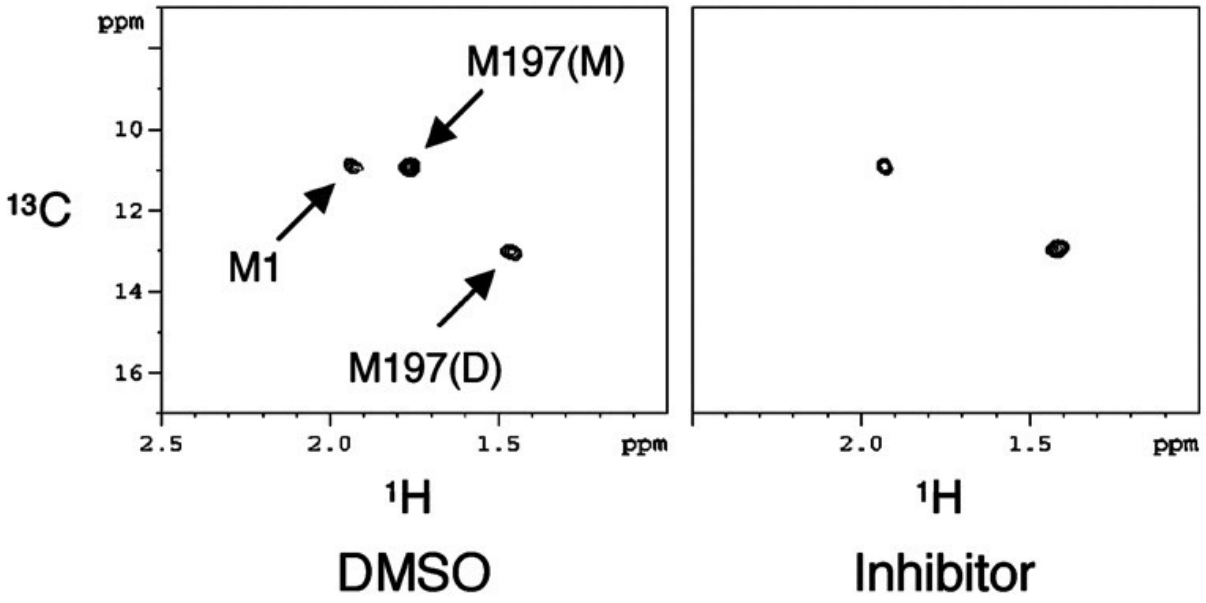
(pH 4.5 - 9.5)

E152Q mutant





Example of slow exchange: monomer-dimer equilibrium



Inhibition of KSHV Pr stabilizes the dimeric conformation

Methionine specific labeling simplifies analysis

Marnett A. B. et.al. PNAS 2004;101:6870-6875