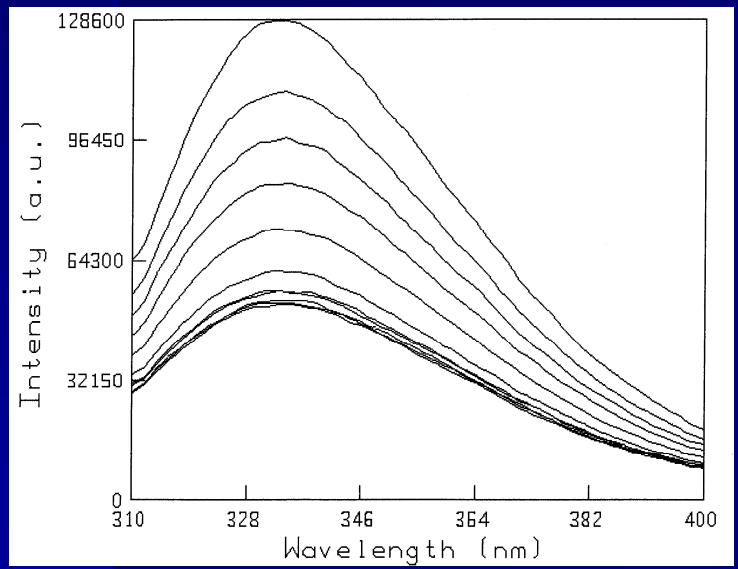
What are the intrinsic flexibilities of an antibody binding site?
C. Irimia '08

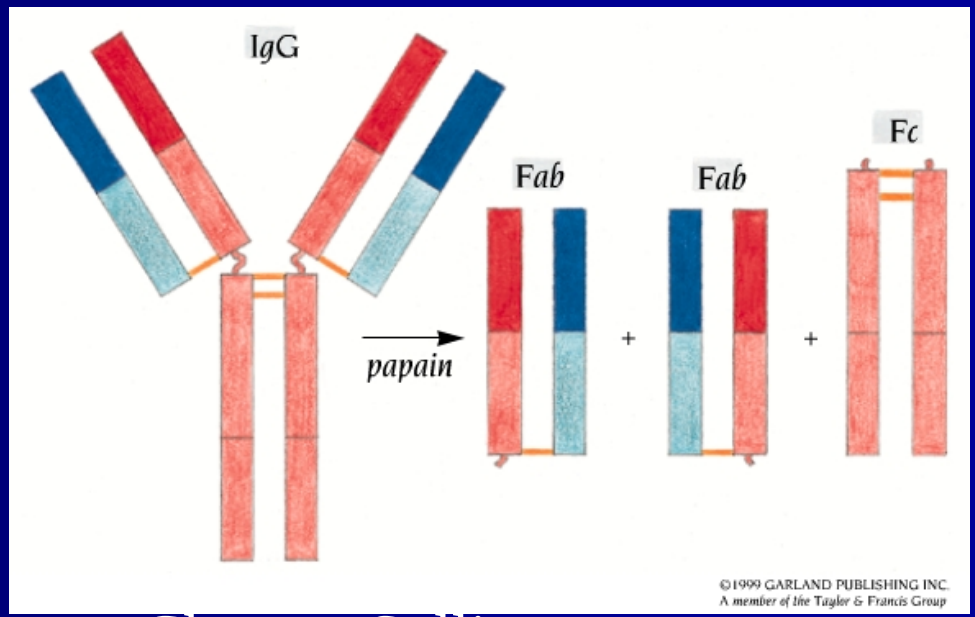
- Antibodies to PLP-Lys^{+} (2006 from JBC)
  - X-ray structures exist with and without hapten
- Compare intact and Fab fragments
- Compare with previous results on Phosphanamidate antibodies
- Computer Modeling of active site empty space

Binding Site Flexibilities

- Trp at binding pockets are quenched when hapten binds.
- But there are 26 Trps in intact protein, only 12 at active site, too much signal!
- Make Fab fragments, reduce that to just 6-7 Trp.

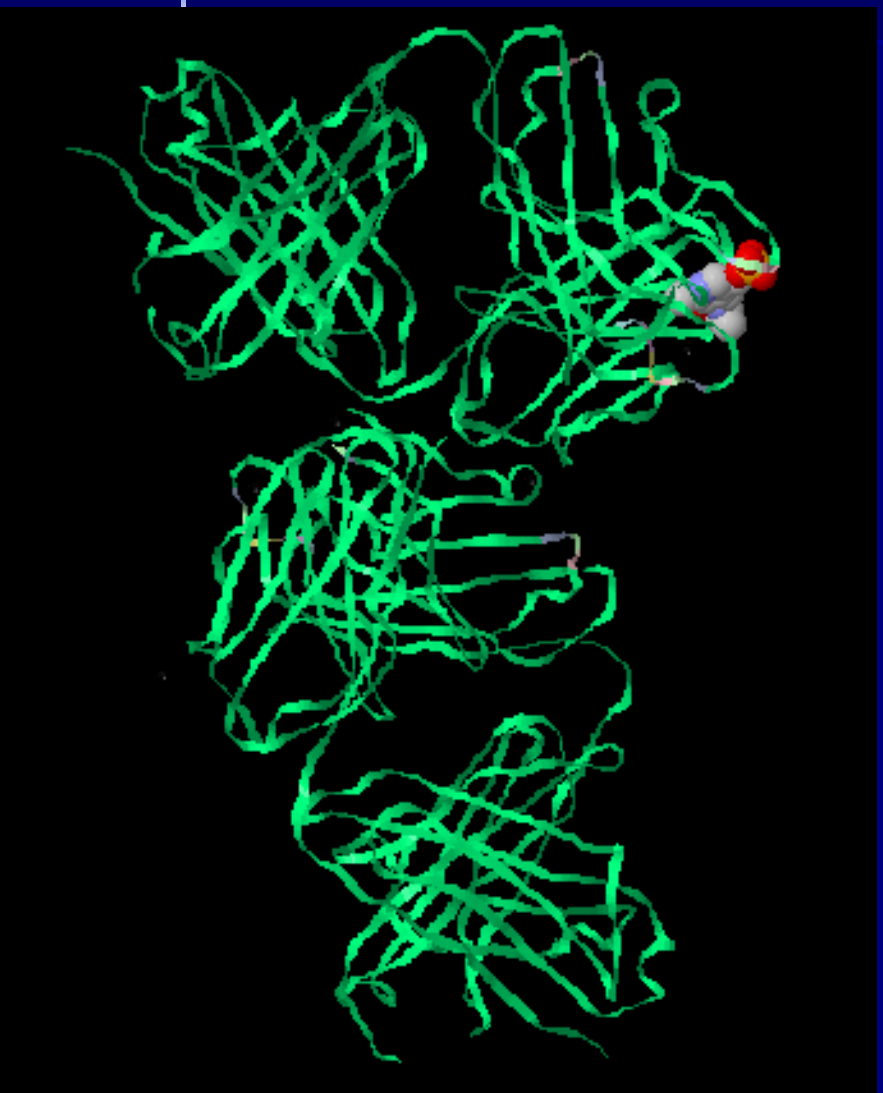


Intrinsic Trp Quench when hapten binds



Chantae Sullivan phosphonamidate antibody

15A9 Structure I overall structure

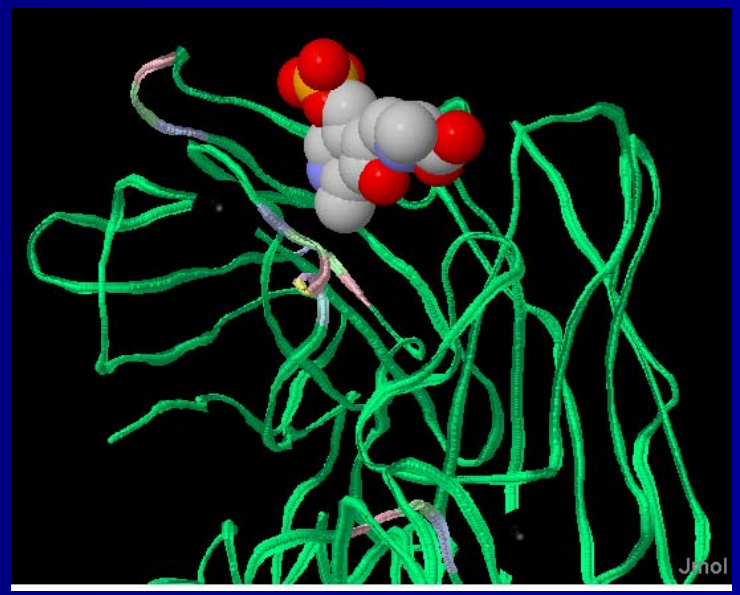


- Fab bound to PLP-Lys antigen X ray picture
- Show constant and variable regions, heavy and light chains, beta secondary structure

- Paper 2006 from JBC with detailed comments on 15A9 structure

15A9 Structure I binding site

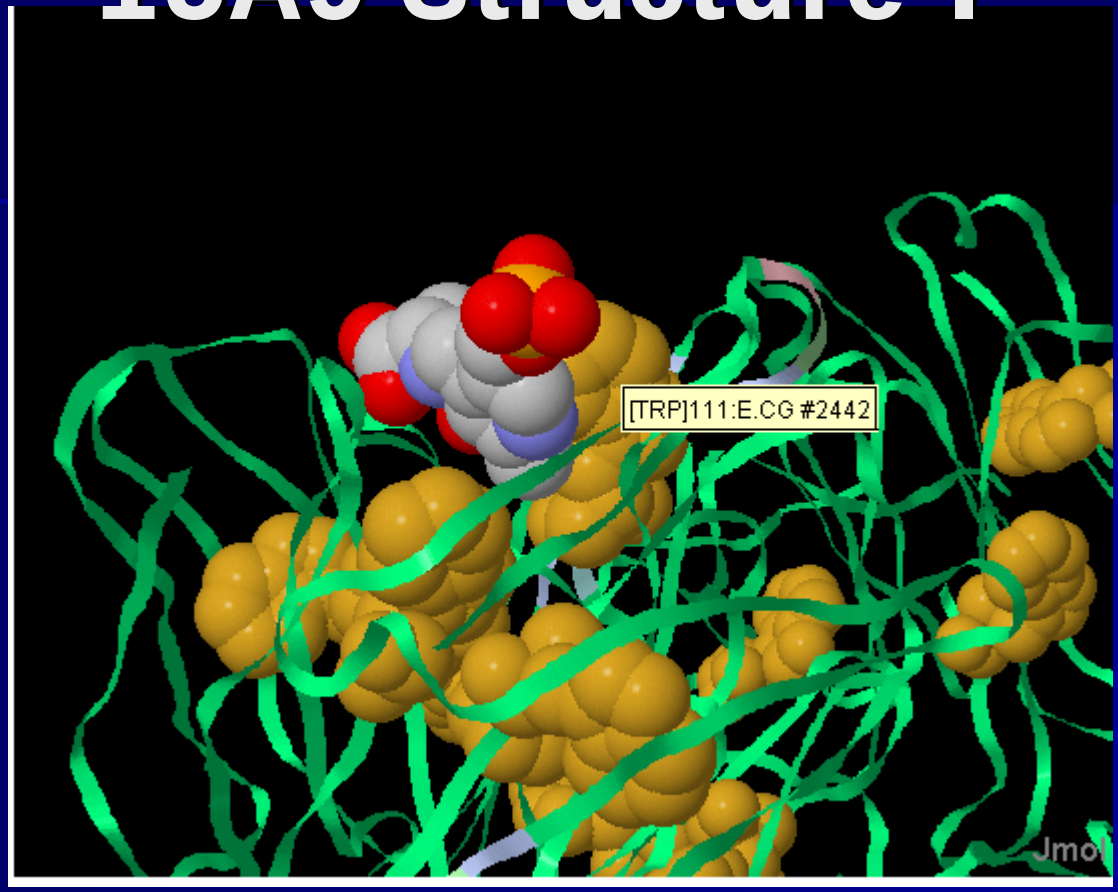
- Fab bound to PLP-Lys antigen X ray picture
- Important residues for binding
- Paper 2006 from JBC with detailed comments on 15A9 structure



15A9 Structure I

- Trp will be spectroscopic reporter:
- This shows that there are 14 Trp (spacefilled and shown in gold) in the Fab

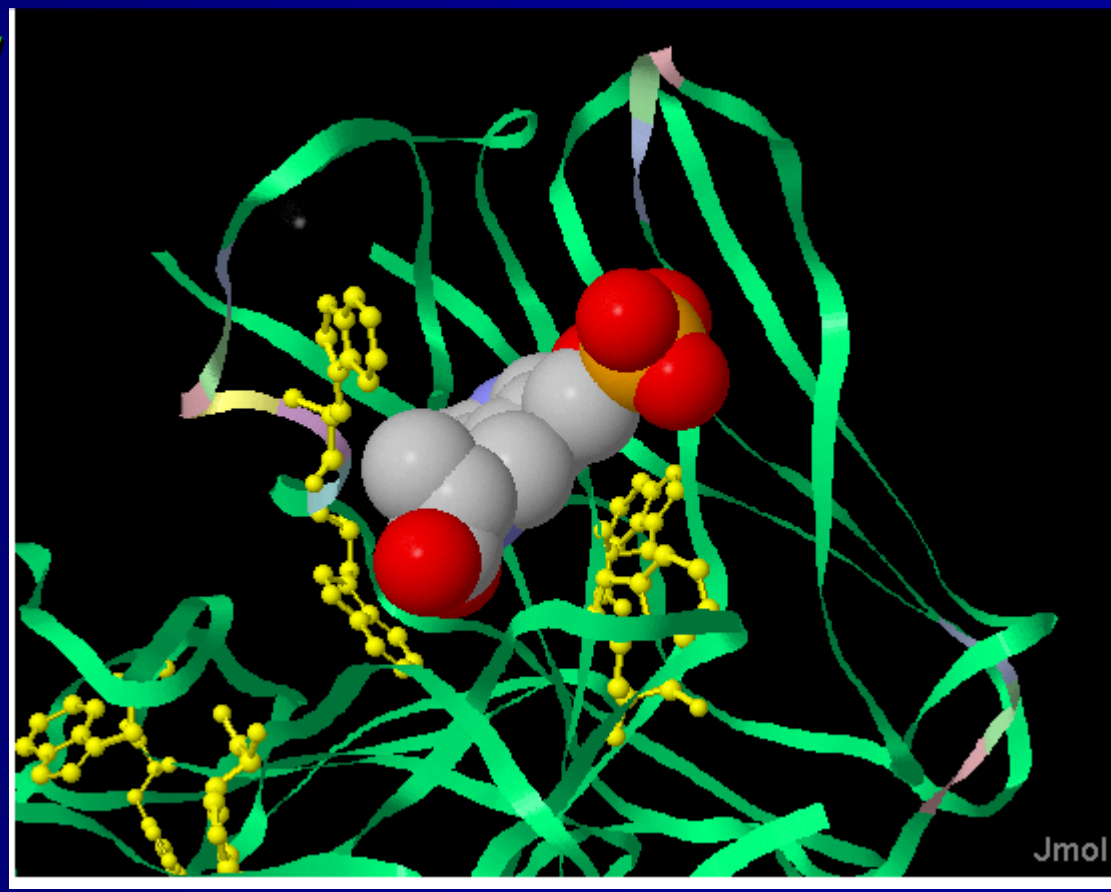
15A9 Structure I



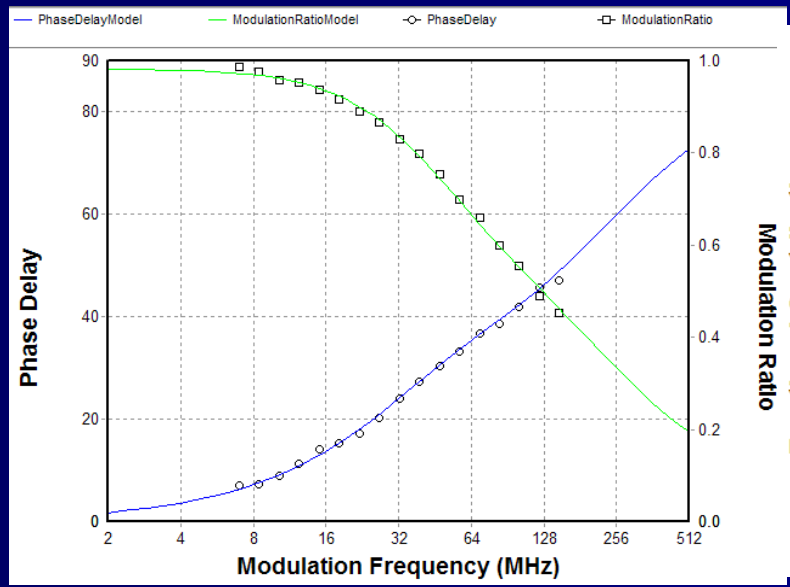
- 4 Trp right at binding site.
- Nearest Trp is highlighted as Trp 111.

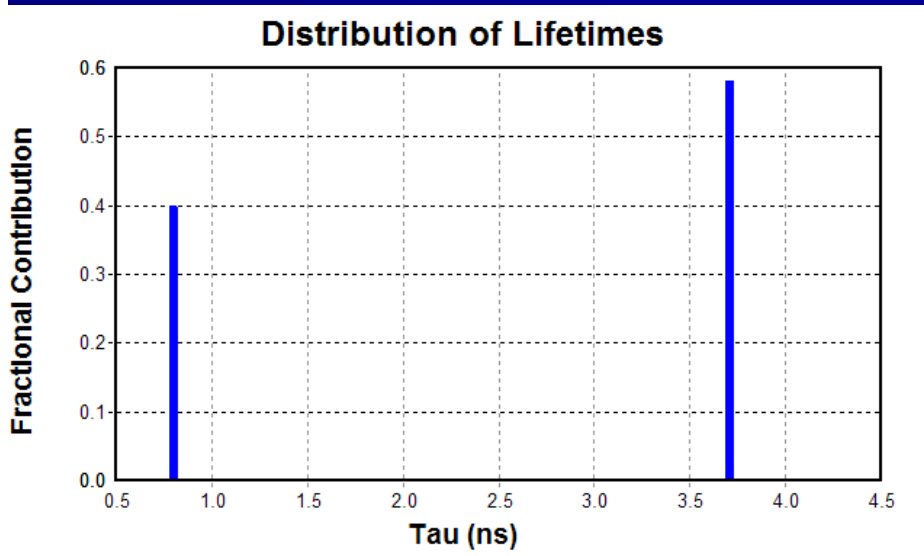
15A9 Structure I nearby trp

- Alternative view showing PLP spacefilled and being embraced by four Trp molecules, with other Trp nearby



Trp Lifetime distributions in 15A9 Fab fragments





Lifetimes (ns)		Contributions		Pre-Exponentials	
Tau1	0.802± 0.04	**f1**	0.403± 0.01	**alpha1**	0.503
Tau2	3.71± 0.08	**f2**	0.579± 0.01	**alpha2**	0.156

15A9 Antibodies, C. Irimia 2008