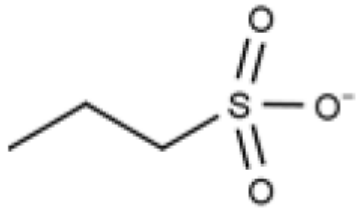
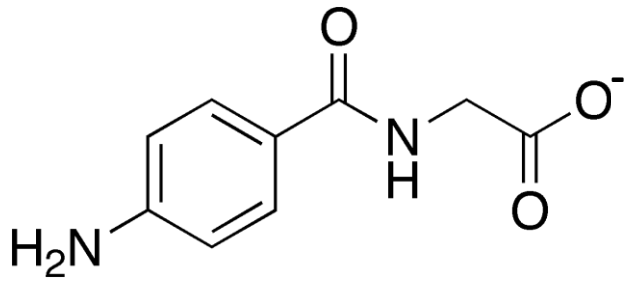
Figure 1: The Ligands employed in NMR experiments to represent the chromatographic resins; (a) SP Sepharose, (b) Nuvia cPrime and (c) Capto MMC ligands.

**a**




**b**




**c**

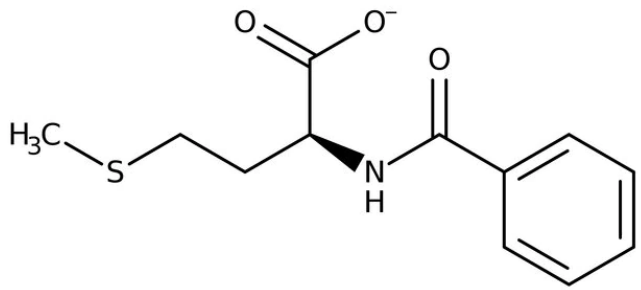


Figure 2: Snapshot from MD simulation of $F_C$ surrounded by Capto MMC ligands in free solution. Protein is shown in a surface representation in grey, water is shown in wireframe and colored based on atom type, and ligands and ions are shown in a licorice representation and colored based on atom type. Color scheme: hydrogen, white; oxygen, red; carbon, cyan; nitrogen, blue; and sulfur, yellow.

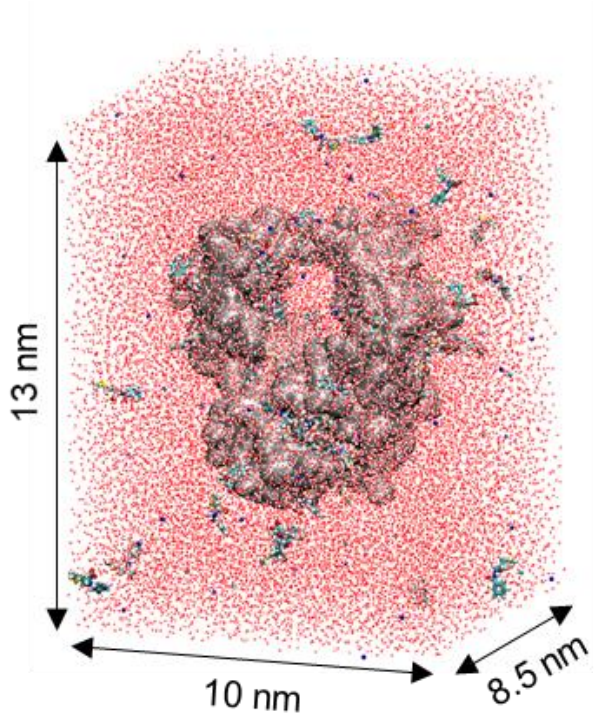


Figure 3: Chromatographic Retention of the $F_C$ domain on single mode SP Sepharose and MM CEX systems. 40 CV linear salt gradient from 0 to 1M NaCl at pH 5.

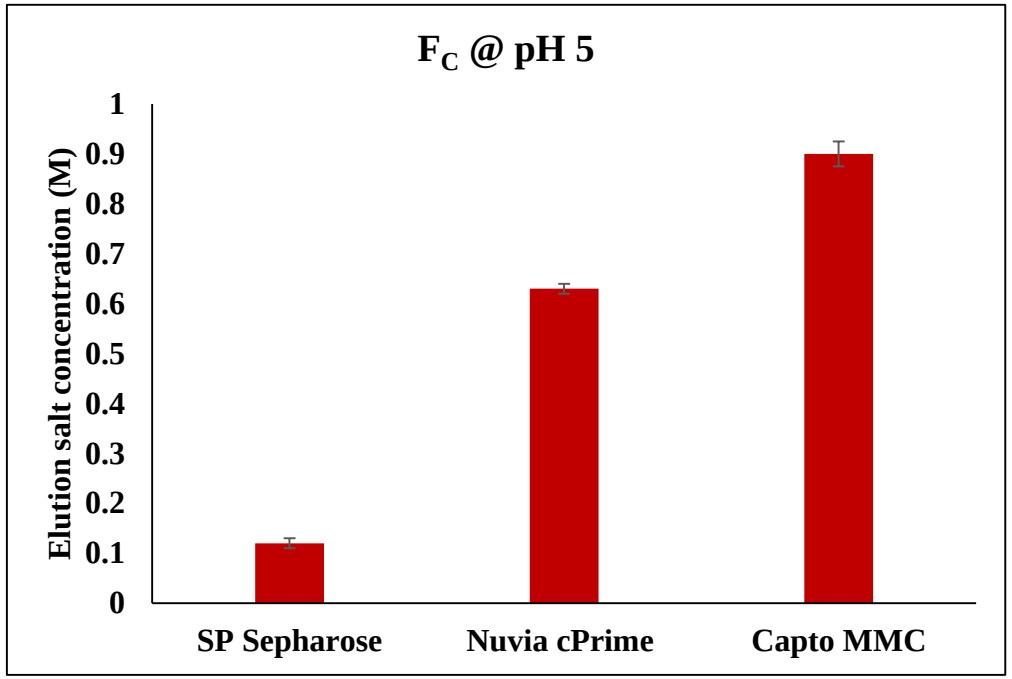
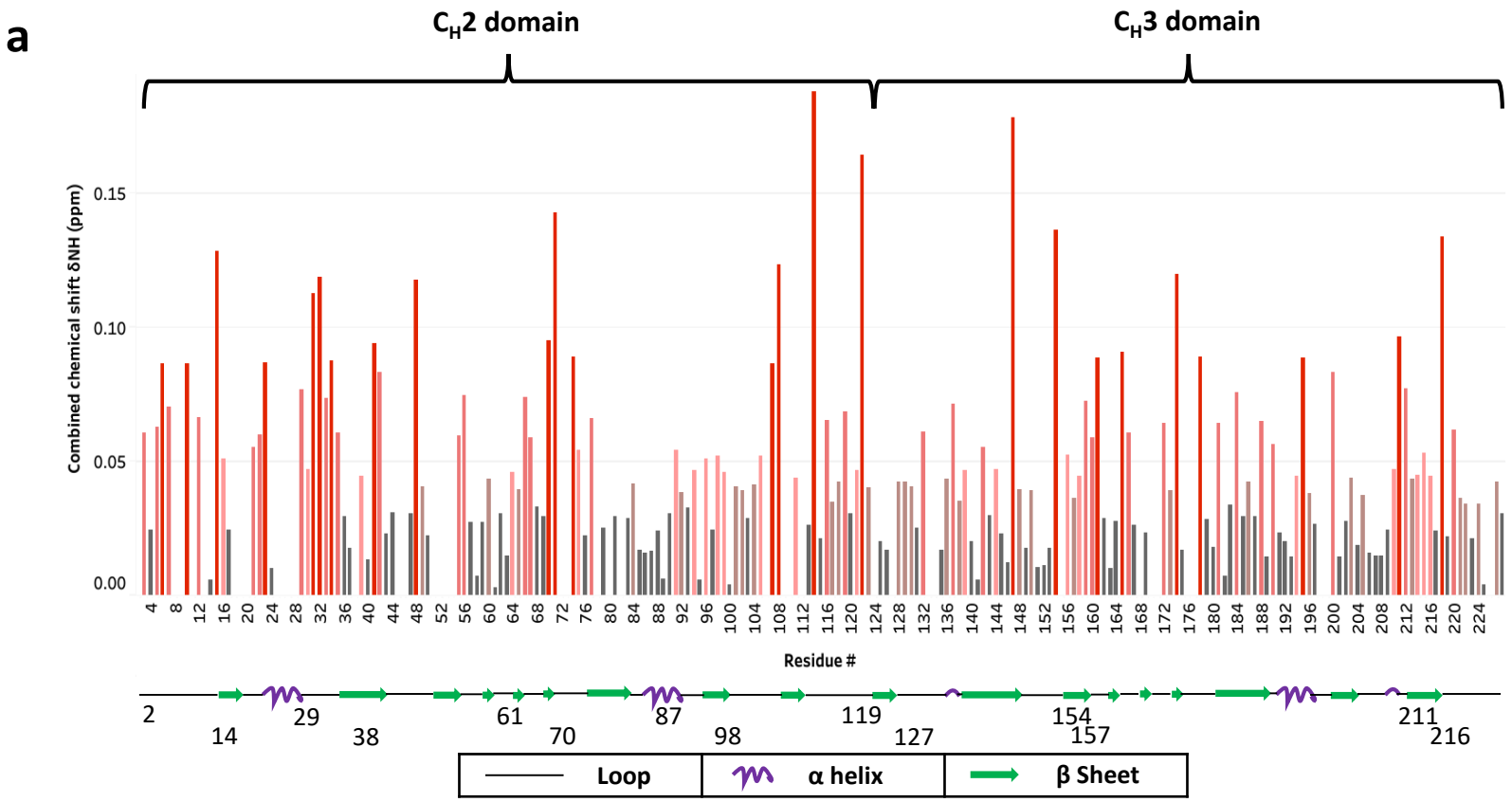


Figure 4: Residue specific ligand-induced changes in combined chemical shift for binding of (a) SP Sepharose, (b) Capto MMC and (c) Nuvia cPrime ligands to the $F_C$ domain. Secondary structure of the $F_C$ domain at the bottom of a. Numbered regions 2-14; 25-38; 61-70; 87-98; 119-127; 154-157; 211-216; represent the loops & α-helices. (Note: different y-axis scale for Nuvia cPrime).



# Figure 4: continue…

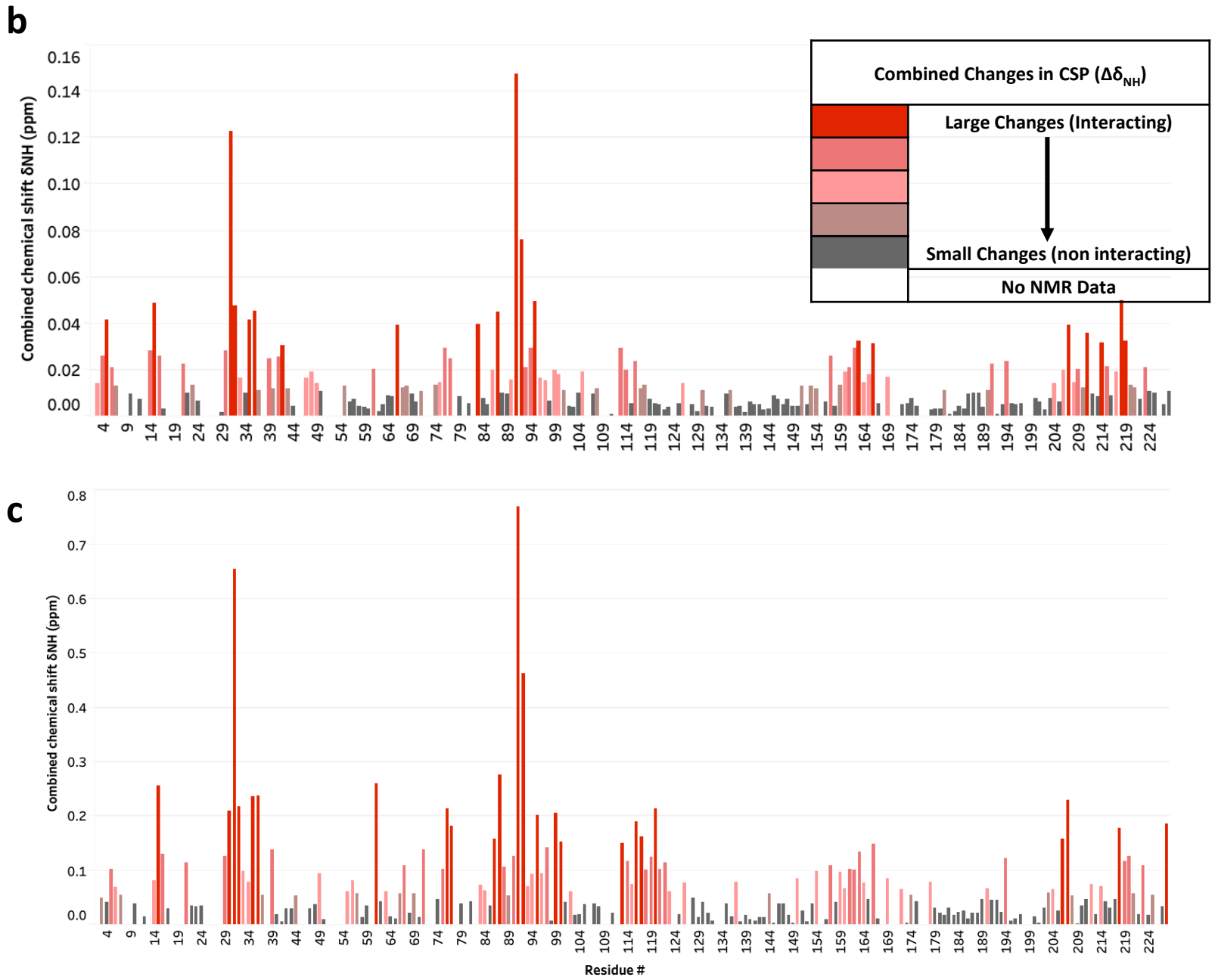
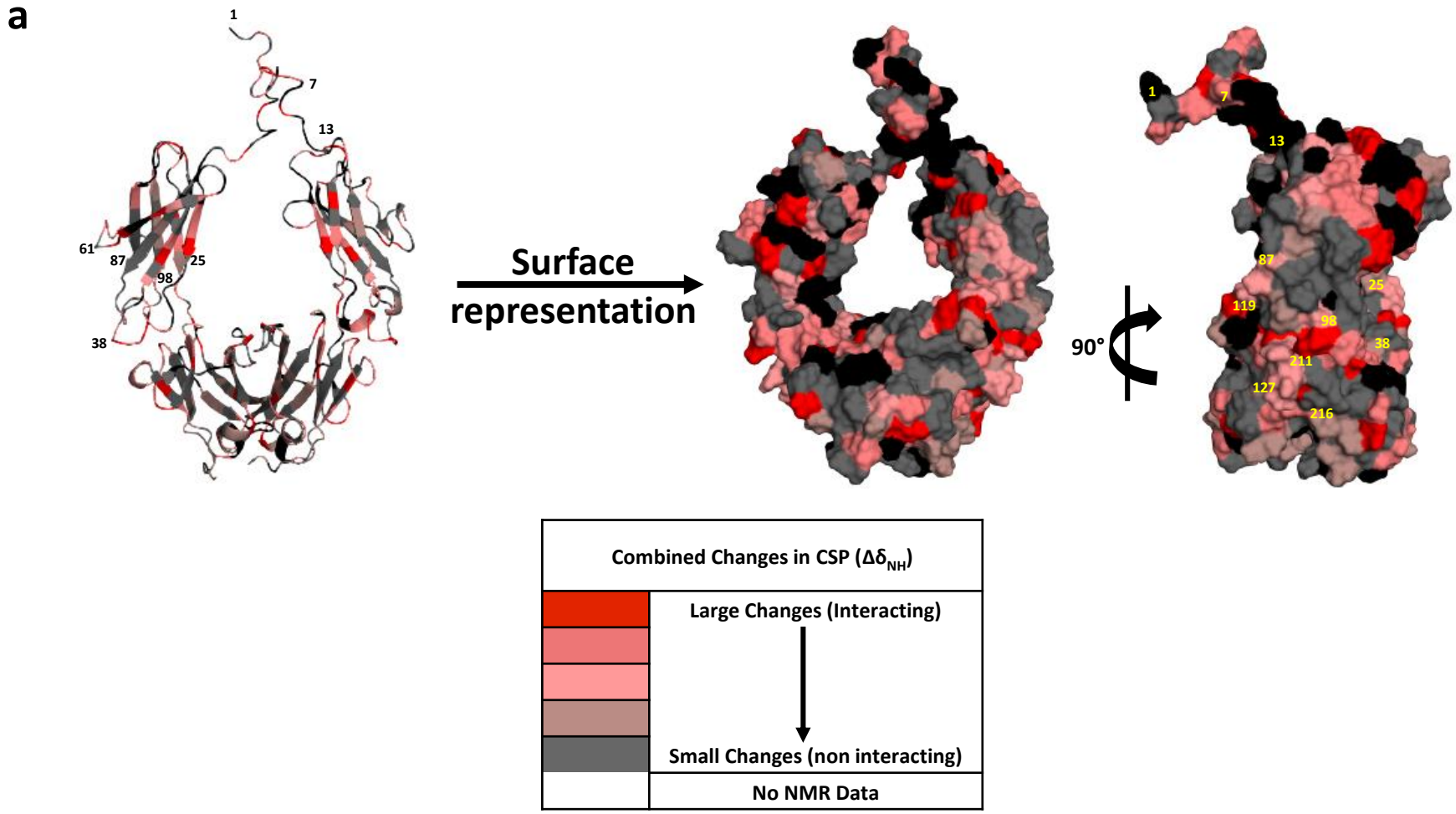
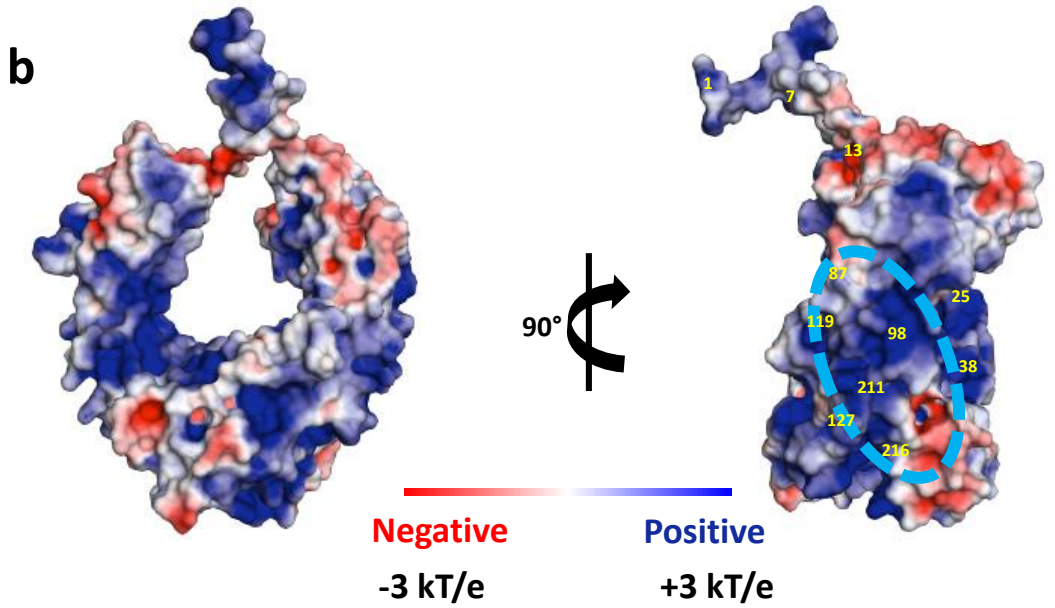


Figure 5: (a) A colored cartoon and surface representation of residue specific ligand induced changes in combined chemical shift upon binding of SP Sepharose ligand to the $F_C$ domain. (b) Electrostatic Potential (EP) map at pH 5 and (c) Surface Aggregation Propensity (SAP) map of the $F_C$ domain. (Note: numbered regions 1-13; 25-38; 87-98; 119-127; 211-216; represent the loops & α-helices)



# Figure 5: continue…



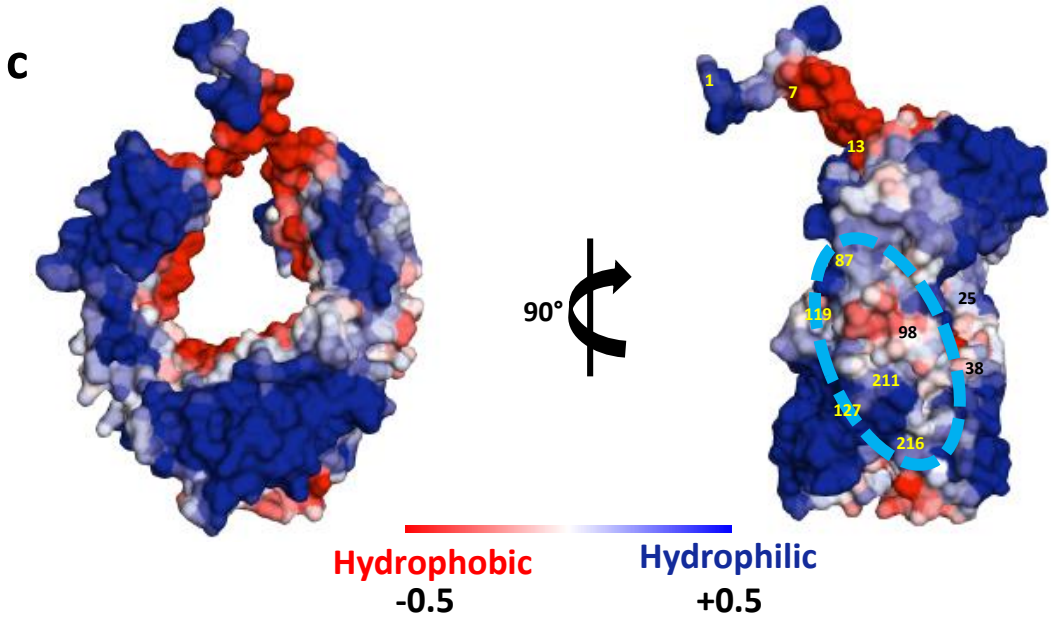



Figure 6: A colored cartoon and surface representation of residue specific ligand induced changes in chemical shift for binding of (a) Capto MMC and (b) Nuvia cPrime ligands to the $F_C$ domain. (Note: numbered regions 1-13; 25-38; 87-98; 119-127; 211-216; represent the loops & α-helices)

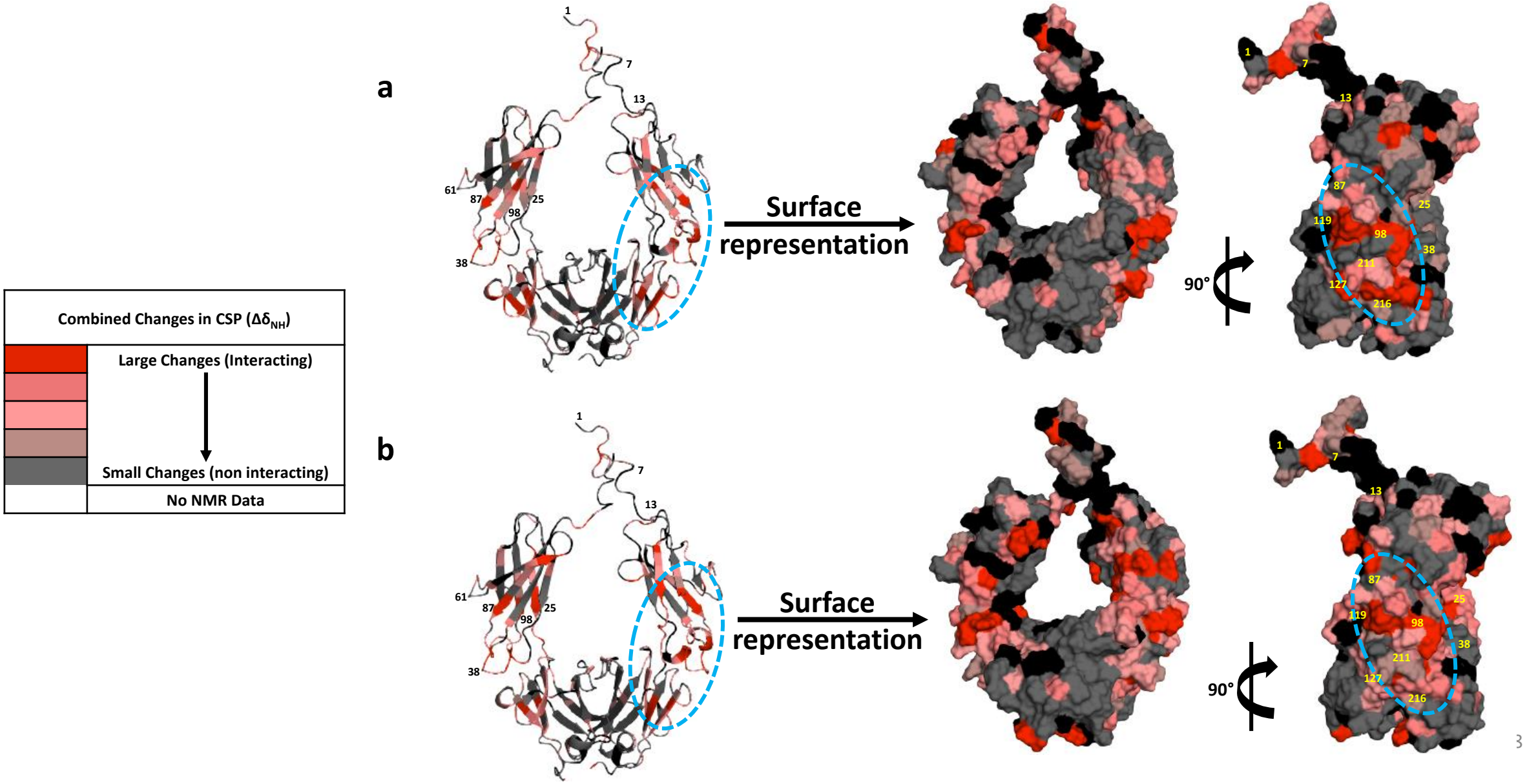


Figure 7: Representative $^{15}$N-TROSY peaks from the Nuvia cPrime titration experiments for residues (a) Aspartate 182, (b) Valine 178 and (c) Histidine 66 at 0 (purple), 5 (magenta), 10(cyan), 20 (green), 40 (pink) mM ligand concentrations

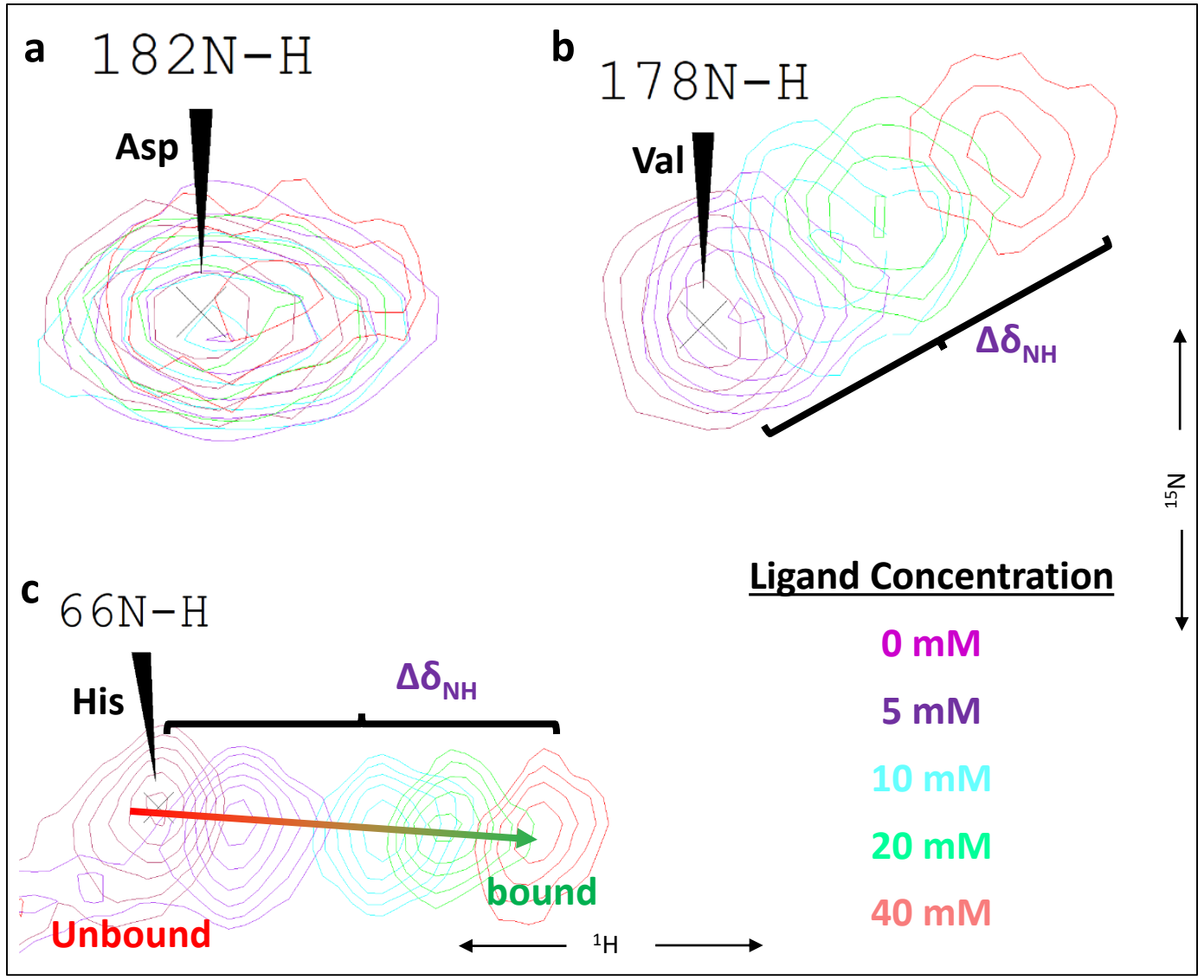
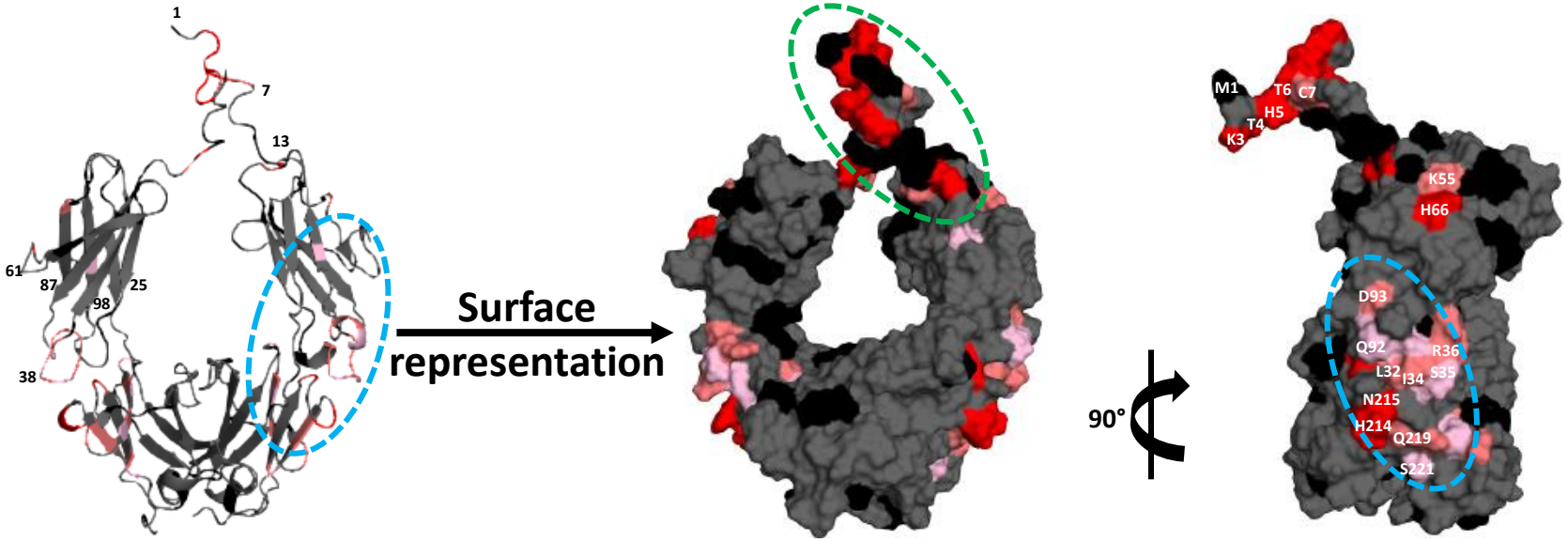


Figure 8: Nuvia cPrime binding sites on $F_C$ domain as determined by NMR with color coded dissociation constant ($K_D$) for non-interacting, grey; and strong, red; intermediate, salmon; and weak, pink; binding residues. Residues located in the hinge and $C_H2$-$C_H3$ interface regions are highlighted in green and blue ellipses, respectively.




| | Dissociation constant ($K_D$) |
|---|---|
| Strong binding | 1.2mM - 10mM |
| | 10.5mM - 30mM |
| Weak binding | 30.5mM - 38mM |
| | No $K_D$ available |
| | No NMR peaks |

Figure 9: Nuvia cPrime binding hotspots on the $F_C$ as determined by MD simulations.

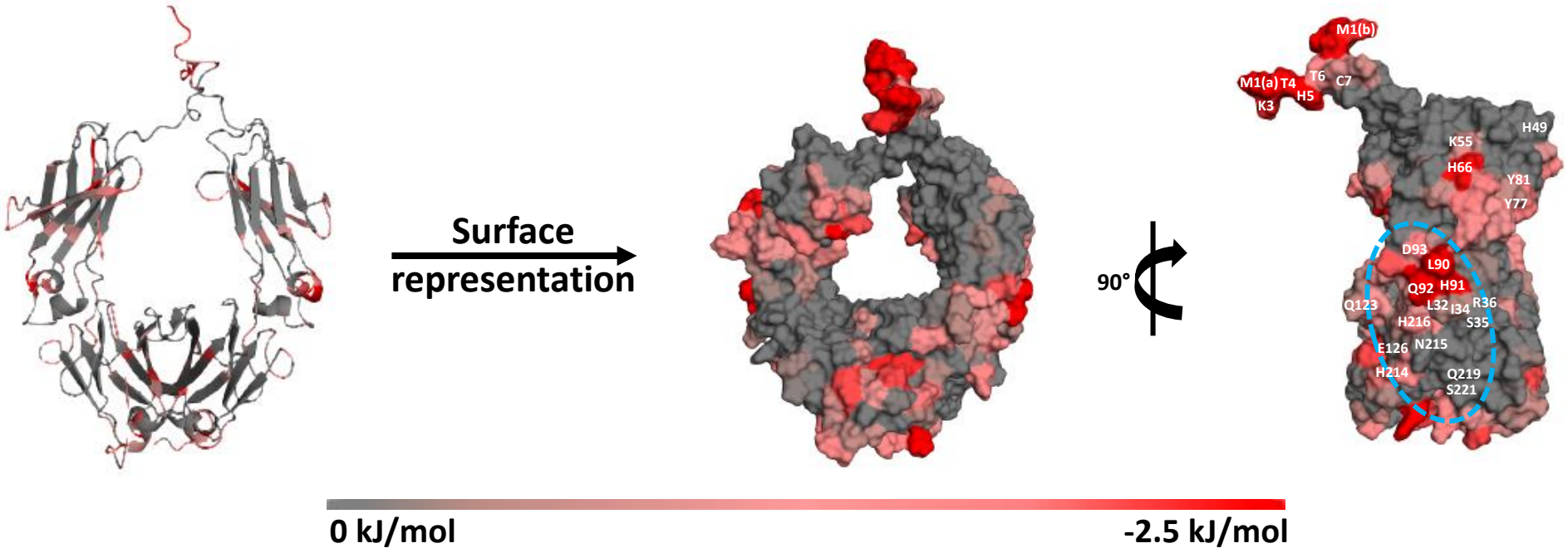


Figure 10: The dual role of protonated histidines in the (a) flexible hinge region and (b) interface of the $C_H2$ and $C_H3$ domains.