2676-Pos Board B106  
Nmr Investigation of Energy Barriers for Hydrogen-Bond Breakage of Protein Side-Chain NH$_3^+$ Groups  
Levani Zandarashvili, Junji Iwahara.  
Dept. of Biochemistry & Molecular Biology, University of Texas Medical Branch, Galveston, TX, USA.  

Hydrogen bonds and ion pairs involving charged side chains are important for protein function. However, their dynamic properties are not well understood. Recently we demonstrated that lysine side-chain NH$_3^+$ groups are strongly pH dependent, with the partially unfolded state dominating at pH values at which the internal Lys residue is charged. In contrast, the L125K protein exhibited fluctuations near Lys-125 on the ms timescale but in a faster regime than the L25K protein, without pH dependence. These results show the extreme degree of coupling between conformational change and proton binding is highly dependent on local structural properties. Computational models attempting to reproduce the properties and pK$_a$ values of buried ionizable groups must be able to represent conformational exchange between native and subglobally unfolded states on ms timescales.

2677-Pos Board B107  
Thumbs II Site Inhibitor AllostERICally Suppresses the Dynamics of HCV RNA-Dependent RNA Polymerase  
Daniel J. Deredge$^1$, Kenneth Johnson$^2$, Patrick Wintrode$^1$.  
$^1$Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD, USA, $^2$Institute of Cellular and Molecular Biology, University of Texas at Austin, Austin, TX, USA.  

Hepatitis C virus RNA-dependent RNA polymerase (NS5B polymerase) is a major target of drug development in the search for an effective treatment of hepatitis C. Various nucleoside analogs (NA) and allosteric non-nucleoside inhibitors (NNI) have been shown to bind NS5B polymerase and inhibit the replication of HCV. NNI inhibitors have been classified based on the drug binding site as observed by crystallography (Thumb I, Thumb II, Palm III, and Palm IV). However, the inherent variability of HCV and the rapid emergence of resistance mutations have highlighted the need for a better understanding of the mechanism of inhibition of NNIs at a molecular level. For that purpose, we have used Hydrogen-Deuterium exchange coupled to mass spectrometry to characterize the dynamics of NS5B polymerase in the presence and absence of an allosteric thumb II site NNi. As expected, we have determined that inhibitor binding to NS5B polymerase causes a significant loss of exchange at the binding site and in regions directly adjacent. Furthermore, we have observed that large regions of MS5B which are distant from the drug binding site display a significant loss in protein dynamics as detected by HD exchange. The observed network of allosterically suppressed dynamics encompasses the fingers, fingers extensions, thumb, C-terminal tail and beta-loop regions which have all been implicated in one of the multiple steps of the reaction cycle (de novo initiation, transition to elongation and processive elongation).

2678-Pos Board B108  
Dysfunctional Conformational Dynamics of Protein Kinase A from R14 Deletion of Phospholamban  
Jonggul Kim$^1$, Larry R. Masterson$^2$, Alessandro Cembran$^3$, Raffaello Verardi$^1$, Lei Shi$^1$, Jiali Gao$^1$, Gianluigi Veglia$^1$.  
$^1$Chemistry, University of Minnesota, Minneapolis, MN, USA, $^2$Chemistry, Hameline University, St. Paul, MN, USA, $^3$Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, USA.  

Ionizable amino acids buried in hydrophobic environments in proteins can titrate with pK$_a$ values. Despite the central role of these amino acids in energy transduction and catalysis, the determinants of their unusual properties are difficult to reproduce with computational approaches. Conformational reorganization of the protein backbone is a key determinant of pK$_a$ values. This reflects the ability of the protein to sample conformational microstates, which is particularly challenging to reproduce computationally. To characterize structural details and timescales of conformational reorganization coupled to ionization of buried groups, NMR spectroscopy was used to identify locations, measure timescales, and determine the extent of pH dependence of conformational exchange in the L25K and L125K variants of staphylococcal nuclease. Lys-25 or Lys-125 titrate with pK$_a$ values of 6.3 and 6.2, respectively. Using relaxation dispersion spectroscopy we observed that the β barrel of the L25K protein fluctuates between the native and an alternate state on the 0.4 to 7 ms timescale. The H$^2$ chemical shifts of the alternate state, assigned using ZZexchange, were less dispersed than those of the native state, consistent with unfolding of the β barrel. The equilibrium between these states is strongly pH dependent, with the partially unfolded state dominating at pH values at which the internal Lys residue is charged. In contrast, the L125K protein exhibited fluctuations near Lys-125 on the ms timescale but in a faster regime than the L25K protein and without pH dependence. These results show the extreme degree of coupling between conformational change and proton binding is highly dependent on local structural properties. Computational models attempting to reproduce the properties and pK$_a$ values of buried ionizable groups must be able to represent conformational exchange between native and subglobally unfolded states on ms timescales.

2679-Pos Board B109  
Dynamical X-Ray Single Molecule Observations of the Molecular Recognition Process in Major Histocompatibility Complex Molecule  
Tomohiro Miyabayashi$^1$, Yufukun Kasai$^3$, Kousuke Yoshida$^1$, Toshihiro Sato$^1$, Yoko Kozono$^1$, Hiroshi Sekiguchi$^1$, Keigo Ikezaki$^1$, Haruo Kozono$^2$, Yuji C. Sasaki$^1$.  

Major histocompatibility complex (MHC) class II molecules have an important role in activities of the immune system. MHC binds peptides from exogenous antigens which is engulfed by endocytosis processes of antigen presenting cells and present it to T cell receptor, then stimulate and activate the immune system. In the peptide-loading process of MHC, it is said that DM which is a homolog of MHC has some activities on that process. The experiment of chromatography using peptide labeled with fluorescein on MHC with DM suggests that DM has a relationship between its activity and the length of peptides. Short peptides tend to be dissociated from MHC, on the other hand, long ones do not. In this research, we used Diffraction X-ray Tracking (DXT) to observe the dynamics of the peptide bound to MHC’s groove with DM as a single molecule. DXT is the method of detecting the detailed motions of the targets for measurement by labeling a gold nanocrystal. The labeled nanocrystals diffract the incident x-rays and we obtained the motions of the target by tracking x-ray diffraction spots. We did experiments at SPRing-8 (BL40XU, JAPAN). From the DXT experiment of observing the motions of two kinds of MHC in MHC’s groove with DM, we obtained the time-resolved (~100 microseconds) motion histograms of peptides. The motions of peptides are obviously different in the peptide bound to MHC’s groove with DM as a single molecule. The DXT is used for detecting the detailed motions of the nanocrystals for measurement by labeling a gold nanocrystal.