domains of unknown function. We also show how minor changes in otherwise highly conserved active sites can significantly affect functionality. There is a growing need for intelligent prediction-based strategies that can tap into our enormous genomic and structural databases and help bridge the gap between sequence and function.

**1183-Pos Board B75**

**Epileptic Seizures-Induced Structural Changes in Rat Spine Bone Tissues: FTIR Microspectroscopic and Chemometric Study**

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Epilepsy is a common serious neurodegenerative disease. Bone disorders due to anti-epileptic drug (AED) therapy in epileptic patients have been reported previously. There is no study in the literature, investigating the independent effect of epileptic seizures on bone tissues. Thus, the side-effects of AEDs on bone tissues could not be differentiated from the effects of the epileptic seizures. The current study provides the first report on clarifying the effects of seizures on bones. The experiments performed on genetically epileptic and healthy rats, give the advantage of studying the effects of seizures alone. Cortical region of spines were studied by FTIR microscopy to investigate the structural and compositional changes in bones. Comparison of FTIR images belonged to the mineral and protein parts of bone clearly showed the difference between healthy and epileptic bone tissues. Mineral content was found to be decreased in epileptic group compared to the healthy control. Although total carbonate content was found to be decreased, B-type carbonate content which substitutes for phosphate groups in the mineral part of bone, was shown to be increased in epileptic group. The organic matrix of bone is mainly composed of collagen proteins whose structure is stabilized by several intermolecular crosslinks. Collagen cross-links ratio was found to be changed critically in epileptic group, indicating an increase in immature crosslinks in the bones of that group. Crystallinity value indicating crystal size was found to be increased in epileptic group compared to the healthy control. Decreased mineral content and collagen crosslinks and increased crystal size and carbonate substitution, imply a severe damage on bone tissues. Moreover, the epileptic and control groups were separated from each other successfully by principle component analysis (PCA) based on the FTIRM data.

**1184-Pos Board B76**

**A Spectroscopic Survey of Substituted Indoles Reveals Effects of $^1L_B$ Transition Stabilization**

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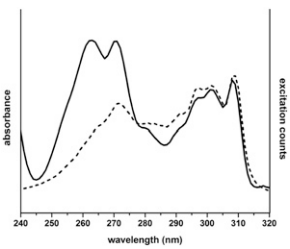
Although tryptophan is a natural probe of protein structure, interpretation of its fluorescence emission spectrum is complicated by the presence of two electronic transitions, $^1L_a$ and $^1L_b$. Theoretical calculations show that a point charge adjacent to either ring of the indole can shift the emission maximum. This study explores the effect of pyrrole and benzyl ring substitutions on the transitions' energy via absorption and fluorescence spectroscopy, and lifetime measurements. The survey of indole derivatives shows that methyl substitutions on the pyrrole ring effect $^1L_a$ and $^1L_b$ energies in tandem while benzyl ring substitutions with electrophilic groups lift the $^1L_a/^1L_b$ degeneracy. For 5- and 6-hydroxyindole in cyclohexane, $^1L_a$ and $^1L_b$ transitions are resolved (5-hydroxyindole absorbance, shown, solid line). This finding provides for $^1L_a$ origin assignment in the absorption and excitation spectra for indole vapor. The 5-hydroxyindole excitation spectrum (dashed line) shows that despite a blue-shifted emission spectrum, both the $^1L_a$ and $^1L_b$ transitions contribute to emission. 10 $^0$ ns fluorescence lifetimes for 5-hydroxyindole are consistent with a charge acceptor-induced increase in the nonradiative rate.




# Enzymes

**1185-Pos Board B77**

**Use Nanomechanical Sensor to Detect Cellulase Activities Including Enzymatic Decrystallization and Hydrolytic Cleavage on Cellulose**

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Cellulase is an interfacial enzyme that catalyzes the hydrolytic degradation of cellulose at the interface between a liquid phase (enzyme) and a solid phase (cellulose substrate). Prior to the hydrolytic cleavage, cellulase utilizes an activity known as enzymatic decrystallization to break up the solid aggregate of cellulose molecules. The activity of enzymatic decrystallization has not been characterized and its mechanism has not been elucidated because very few existing experimental approaches are able to examine interfacial enzymatic activity on solid substrates. Here, we report the development of a novel strategy for the real-time detection of cellulase activities including enzymatic decrystallization and hydrolytic cleavage on cellulose with the use of a nanomechanical sensor in a microcantilever. We present both kinetic and physical evidence to support the decrystallization as a kinetically viable step of cellulose hydrolysis by cellulase. To our knowledge, this is the first use of a nanomechanical sensor to study mechanistic enzymology and heterogeneous enzymatic catalysis that involves a solid substrate. This nanomechanical sensor-based approach will help obtain a comprehensive understanding of cellulase actions on cellulose, which would be essential to the success of the development of new cellulases with enhanced efficiency for biofuels production.

**1186-Pos Board B78**

**Single Enzyme Studies Reveal the Existence of Discrete Functional States for Monomeric Enzymes and How they are "Selected" upon Allosteric Regulation**

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Allosteric regulation of enzymatic activity forms the basis for controlling a plethora of vital cellular processes. While the mechanism underlying regulation of multimeric enzymes is generally well understood and proposed to primarily operate via conformational selection, the mechanism underlying allosteric regulation of monomeric enzymes is poorly understood. Here we monitored for the first time allosteric regulation of enzymatic activity at the single molecule level (1). We measured single stochastic catalytic turnovers of a monomeric metabolic enzyme (Thermomyces lanuginosus Lipase) while titrating its proximity to a lipid membrane that acts as an allosteric effector. The single molecule measurements revealed the existence of discrete binary functional states that could not be identified in macroscopic measurements due to ensemble averaging. The discrete functional states correlate with the enzyme's major conformational states and are redistributed in the presence of the regulatory effector. Thus, our data support allosteric regulation of monomeric enzymes to operate via selection of preexisting functional states and not via induction of new ones.

(1) Hatzakis, N. S.; Wei, L.; Jørgensen, S. K.; Kunding, A., H.; Bolinger, P-Y.; Ehrlich, N.; Makarov, I.; Skjot, M.; Svendsen, A.; Hedegård, P.; Stamou, D. (2012). Single Enzyme Studies Reveal the Existence of Discrete Functional States for Monomeric Enzymes and How They Are "Selected" upon Allosteric Regulation. J. Am. Chem. Soc.134 (22), 9296-9302.

**1187-Pos Board B79**

**Backbone 1H-13C-15N NMR Assignments and Ligand Binding Study of OMP Synthase from Saccharomyces Cerevisiae**

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Catalysis in OMP synthase (orotate phosphoribosyltransferase, EC 2.4.2.10) is coupled to the unstructured-to-structured transition of a 10-residue peptide loop. OMP synthase from yeast is a small, homodimeric (49 kDa) and highly stable domain-swapped enzyme that catalyzes the formation of the UMP