site, identify sequence preference, and possibly identify proteins cleaved by the protease. To achieve protease substrate identification, we have created peptide conformer libraries and docked peptides into crystal structures and homology models of several proteases. Understanding the substrate spectrum of these proteases can facilitate the design of peptide mimics that can be developed into inhibitors. In addition to identification of substrates, we are taking a fragment-based approach to design new inhibitors. Drug-like fragments from multiple compound libraries are docked into the protease active site and top hits are later screened in crystal soaking experiments and in parasites. An *in silico* approach followed with *in vitro* confirmation and *in vivo* evaluation is a starting point for developing new anti-malarial therapies.

1184-Pos Board B94
Loop Conformation Prediction through Saddle-Point Search Simulations using the Art Nouveau Method and OPEP Simplified Potential
Jean-Francois St-Pierre, Normand Mousseau.
Three dimensional structures of proteins can often be obtained by experimental methods such as x-ray diffraction crystallography or nuclear magnetic resonance, or through homology modeling on proteins that share sufficient sequence conservation and for which a structure is known. Problems arise when a part of a protein is too floppy to be captured by experimental methods or when its sequence is not available for homology modeling in the homologous protein structure. For these loops, structure prediction can be obtained using one of two classes of algorithms. The first family of methods is known as database searching methods and has obtained significant success with loops of at most 15 amino acids. These methods which build a loop structure from library of loops or fragments are relatively fast but are limited in the size of loops they can predict with low RMSD error. The second family of prediction methods is called ab initio and predicts structure by exploring the energy landscape of the loops from a starting non-native structure. In essence, they solve the problem of protein folding for a fragment of the structure to predict and have a computational cost proportional to the level of approximation of their energy potential. We adapt our Monte Carlo energy landscape exploration method ART nouveau to predict loop structures of increasingly larger size. Coupled with the coarse-grained potential OPEP, ART nouveau has been used to study the folding pathways of proteins of up to 60 amino acids and aggregation of amyloid fibrils forming peptides. Comparing ART nouveau results on 20 sequences recently characterized we show that our algorithm is competitive.

1185-Pos Board B95
NMR Structure Determination by Conformational Space Annealing
Jooyoung Lee, Jinhyuk Lee, **Keehyoung Joo**, Jinwoo Lee.
We have carried out numerical experiments to investigate the applicability of global optimization method to the NMR structure determination. Since the number of NMR observables is relatively small in the early stage of NMR structure determination process and long range NOE observables are difficult to obtain, advanced sampling techniques are greatly in need to generate valid NMR structures from a small number of experimental restraints. By utilizing conformational space annealing method, we have determined solution NMR structures from NOE distance and backbone dihedral restraints. Several solution NMR structures are determined starting from fully randomized conformations. We have evaluated them by measuring the qualities of determined structures, such as structure convergence of ensemble, Ramachandran preferences, clash scores, and the total NOE violation. These qualities are compared to those from the corresponding PDB structures. When we applied the method to recent PDB deposits of 120 proteins ranging from 50 to 261 residues, we were able to generate protein structures which are superior to PDB structures in all four criteria for about 80% of the cases.

1186-Pos Board B96
Design and Analysis of De Novo Collagen-Like Heterotrimers using Charge Repulsion-Based Negative Design
Mihir Joshi.
Collagen, the most ubiquitous protein in the body, is the primary component of the extracellular matrix (ECM). The collagen molecule's folding mechanics have not yet been fully elucidated, which has hindered the progress of *de novo* synthetic collagen matrices and biomaterials.
Our lab seeks to construct rationally-designed collagen-related peptides (CRPs) for structural mimetic studies, allowing for further analysis of the interactions guiding the self-assembly of collagen's signature triple helix. We have designed a set of three polypeptides A, B, and C, with positively charged arginines placed at the N-terminus, center, and C-terminus respectively. The rest of the polypeptide is made of highly stable Pro-Hyp-Gly repeats. The design was conceived such that an A-B-C heterotrimer will be favored due to the presence of repulsive charges on adjacent positions in all competing products. These peptides, after being combined in solution, were tested for the presence and stability of formed triple helices. Consistent with previous work, CRP folding may be non-cooperative and nucleated, processively leading from the C-terminus to the N-terminus. Interestingly, repulsive interactions do not effectively determine specificity of assembly.
To further understand the role of unfavorable interactions in specificity of assembly, we are constructing negatively-charged aspartate analogs of the current system. Structural modeling suggests aspartates should have a larger impact on stability and specificity of assembly.

Enzymes/Heme Proteins I

1187-Pos Board B97
Synthesis, Crystal Structure, Electrochemical Studies and Artificial Tyrosinase Activity of a New Designed Homobinuclear Copper (II) Complex
Hossein Derakhshankhah, **Ali Akbar Saboury**, Adeleh Divsalar, Hasan Mansouri-Torshizi.
Dicopper proteins, like hemocyanin, tyrosinase and catechol oxidase, contain a coupled binuclear metal center, which plays important roles in biology. Hence, in the present investigation we decided to design and synthesize a new class of homobinuclear copper (II) complex (with formula [(dien) Cu (μ-1,6-DAH) Cu (dien)]$(NO_3)_4$, where μ-1,6-DAH=1,6-diaminohexane binuclear). Characterizations of this complex were investigated using X-ray crystallography, X-ray powder diffraction (XRD), thermal gravimetric analyses (TGA) and cyclic voltametry (CV) methods. Also, the catalytic activity of the complex checked as an artificial tyrosinase and the optimum condition for artificial enzymatic activity was determined using kinetic assays**.** X-ray crystallography data showed that this complex has been crystallized in the monoclinic system, space group P 21/n, with a= 8.0297(8) Å, b= 12.4937 (14) Å, c=15.3786(15) Å and z=2. Each copper (II) has a square based pyramidal coordination geometry with four N atoms building the basal plane (three from dien and one from μ-1,6-DAH) and a nitrate group occupies the apical position. XRD results of this complex represented a single crystal, which obtained as a mono-phase. Thermal stability and thermal gravimetric analyses (TGA) represented that the complex is stable up to 245°C. Also, artificial tyrosinase activity of the complex in the base of formation of quinon as a product showed that this complex has a maximum cathecolase activity at pH 8, temperature of 40°C and ionic strength of 50 mM.

1188-Pos Board B98
Role of Histidine-932 of Human Mitochondrial DNA Polymerase in Nucleotide Discrimination and Inherited Disease
Dipanwita Batabyal, Jessica L. McKenzie, Kenneth A. Johnson.
The human mitochondrial DNA polymerase (POLG) is nuclearly-encoded and is solely responsible for the replication and repair of the mitochondrial genome. The progressive accumulation of mutations within the mitochondrial genome is thought to be related to ageing, and mutations in the POLG gene are responsible for numerous heritable disorders including progressive external opthalmoplegia (PEO), Alpers syndrome and Parkinsonism. Here we investigate the kinetic effect of H932Y, a mutation associated with PEO. Mutations H932Y and H932A reduce the specificity constant governing correct nucleotide incorporation 150- and 70-fold respectively, without significantly affecting fidelity of incorporation or the maximum rate of incorporation. However, this leads to only a twofold reduction in rate of incorporation at a physiological nucleotide concentration (~100 M). Surprisingly, incorporation of T:T or C:T mismatches catalyzed by either H932Y or H932A mutants was followed by slow pyrophosphate release (or fast pyrophosphate rebinding) Also, H932Y readily catalyzed incorporation of multiple mismatches, which may have a profound physiological impact over time. H932 is thought to contact the beta phosphate of the incoming nucleotide, so it is perhaps surprising that H932Y appears to slow rather than accelerate pyrophosphate release.

1189-Pos Board B99
Measuring the Enzymatic Activity of Clinically Important Proteins in Single Cells
Christopher E. Sims, Nancy L. Allbritton, Dechen Jiang, Shan Yang, Angie Proctor, Ryan Phillips.
Molecularly targeted therapies are at the forefront of clinical science, and are expected to lead to personalization of medical treatments for each patient. Most such therapies are directed at inhibiting specific signal transduction enzymes or pathways, thus creating a critical need for assays capable of measuring the activities of these proteins in disease models and in patient samples. The ability to measure relevant enzyme activity in primary cell samples at baseline and/or after treatment would provide the ability to tailor patient therapy based on aberrant signal transduction, validate mechanisms of resistance in patients, and would offer an invaluable pharmacodynamic tool to assess whether