

its proposed role in the binding of multiple metal ions and in their transportation to a silver ATPase. My goal is to isolate the SilE protein and attempt to characterize its physical and metal binding properties. My goal co-insides with the overall project goal, which is to understand the mechanism of the Sil system and eventually find a way to circumvent its silver resistance.

68 Developing Chemical Crosslinking and Mass Spectrometry for Large Protein Structure Determination

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Dynein heavy chain (HC) is a 530 kDa subunit of the microtubule-associated, motor protein dynein. Dynein HC is a potential cancer target because of its vital role in the cell cycle, yet little is known about its structure. While the amino acid sequence of dynein HC has been determined, an understanding of the spatial configuration of the peptide backbone is lacking. However, the size of dynein makes traditional methods for tertiary structure determination impractical. Recent studies have demonstrated an alternate method based on amino acid specific cross-linking and mass spectrometry. A chemical cross-linking reagent features dual lysine reactivity and covalently binds to primary amines separated by 5-10Å. After cross-linking the protein is digested and peptide fragments are purified and sequenced by liquid chromatography mass spectrometry (LC-MS). The distance constraints between lysine residues provide initial parameters for computational structure determination.

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Contralateral Compensation in Stair Climbing After Unilateral Ankle Arthroplasty

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Performance of stair climbing after total ankle arthroplasty is an important measure of patients' abilities. Fourteen patients wearing low cut shoes ascended and descended a set of four steps one year after unilateral ankle arthroplasty with a semi-constrained system. The functional range of motion (ROM) at the knee and ankle were computed by gathering kinematic data via PEAK5 motion analysis system. The angle between the foot and direction of motion (progression angle) was calculated by inputting the kinematic data in a MATLAB program specifically written for this experiment. Progression angle measures the compensation achieved by external rotation of the foot. After three trials of data were collected for each patient, looking at both operated and un-operated sides, results suggested that increased knee flexion compensated for loss of ankle movement during ascent, while an increase in progression angle was used to compensate in descent. Patients' contralateral abilities may be necessary for a successful total ankle arthroplasty.

70

Synthetic Studies Toward the Generation of Deoxystreptazolin Derivatives

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Streptazolin is a natural product with anti-biotic and anti-fungal activity, and various derivatives have been synthesized which show increased activity. Our research focuses on developing a rapid synthesis of deoxystreptazolin derivatives. This strategy centers around the selective addition of an allyl borane to an aldehyde followed by a Rhodium-catalyzed cyclization to generate the core structure of deoxystreptazolin. This core can then be derivatized to numerous deoxystreptazolin analogues.

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Analysis of the NSF REU Chemistry Applicant Pool: What is the Demand for Undergraduate Research?

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On behalf of the NSF's REU Leadership Group in Chemistry, we have gathered aggregate information on applicants to Chemistry REU sites for three different years (2001, 2003-04). The focus of our project is to analyze these statistics to gain insight into the demands and needs for summer REU positions (i.e., a "demand survey"). Among the issues we are analyzing the data for are similarities and differences between applicants from Research Intensive

Institutions (RIIs) and Primarily Undergraduate Institutions (PUIs), how broadly students apply, and whether offers are made to the same small fraction of applicants. In addition to collecting "short form" data from the majority of sites, more extensive demographic data was collected from a small number of sites for a detailed look at a portion of the applicant pool. We anticipate that our analysis can be used by the NSF as they allocate their limited resources among their many programs.

72

Testing the New Long-Range Model for Membrane Protein Folding

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Understanding the folding process of membrane proteins is a fundamental biomedical challenge that has lagged behind that of soluble proteins due to the hydrophobic nature of the membrane proteins. The shape a protein takes after undergoing the folding process determines the function of the protein. A new model for membrane protein folding has been proposed that states that long-range interactions between amino acids from both loop and transmembrane helices take place during the very early stages of folding, before and during the formation of helices. To test this model, we use a computational method to predict protein folding nuclei from native state structures that is based on a constraint network model of freely rotating rods. This method uses an all-atomic analysis of the rigidity and flexibility of protein structures, which includes specific hydrophobic, polar and charged interactions. The objective is to test the validity of the new model.

73

Modeling Transcription Factor Binding Sites with Dependencies

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Protein-DNA binding is essential to transcriptional control, a key mechanism of the gene expression regulation. In general, computational models have the potential to efficiently model transcription factor binding sites (TFBSs), though the complexity of the interactions in some cases pose a major challenge. Basic models consider each position of a TFBS to contribute independently to protein binding. This project looks into modeling dependencies between positions in the TFBS. The use of mutual information content will elicit dependencies between different positions in a collection of binding sites for a given transcription factor. The significance of these values is determined by comparing known MIC values to a distribution of semi-randomly generated values. Scoring methods will be used considering dinucleotide and trinucleotide dependencies as well as independent positions to create a more accurate TFBS model. Finally, the accuracy of these models are tested compared to strictly independent models.

74

Stochasticity in NF- κ B Regulation

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The nuclear factor κ B (NF- κ B) family of transcription factors is important in the expression of many genes, including several involved in the immune response. A model was recently proposed that approximates this pathway using ordinary differential equations. The basic processes included in that model are those of NF- κ B and its activator, I κ B (IKK), as well as its inhibitors, A20 and I κ Ba. Because this model uses ordinary differential equations, there is no inclusion of noise and fluctuations which often have great effects on biological systems. We

converted the ordinary differential equations into stochastic differential equations, allowing us to study the possible implications of noise on the deterministic model by analyzing the qualitative differences in the dynamics of the system.

75

Weighted Probability in Absolute Entropy Calculation of a Lattice Model

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The absolute free energy, F , is a criterion of stability, thus calculating F is mandatory for defining the native structure of a protein. $F=E-TS$, where E is the average energy, T is the absolute temperature and S is the absolute entropy. In simulations it is easy to calculate E but very difficult to obtain S , hence F . A new method for calculating S suggested recently by Meirovitch's group is tested as applied to a self-avoiding-walk on a square lattice, which constitutes a simplified model for a denatured protein. Understanding protein folding is essential for future applications of molecular medicine and disease research.

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Solvent entropy estimation at the nanosecond timescale in molecular dynamics simulations

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Solvent entropy is recognized as the driving force of protein folding, and we propose that native folds are the ones that maximize solvent entropy. Quantitative descriptions of this process have been proposed, but current experimental methods do not have the detail or speed to probe the exact changes in solvent entropy during folding. This project uses the molecular dynamics program CHARMM to simulate polypeptides solvated by explicit water. An index of solvent entropy is monitored over the course of the simulation. This project is a test bed for developing more atom-realistic quantitative solvent-entropy simulations. Validation of this method can be used to develop new folding simulations driven by solvent entropy. Such accurate simulations can ultimately lead to protein structure prediction from amino acid sequence alone.

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Homology Modelling of Melatonin Receptors

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G-protein coupled receptors (GPCRs) are targets for 50% of all existing medications, and are thought to be in equilibrium between an active and inactive state. GPCRs are characterized by the presence of seven transmembrane helices composed of hydrophobic sequences; however, the actual sequence identities vary greatly. The melatonin receptors, specifically the MT1 and MT2 receptors, are of particular interest because their structures have not yet been determined. MOE and Modeller were used to both align the sequences with a template sequence, bovine rhodopsin, and then create a model using its structure. Two specific motifs of the melatonin receptor are of interest: the "aromatic cluster" motif and the "N(P)XXY" motif in transmembrane 7, both of which are found in GPCRs. Similarities and contrasts between GPCRs/Melatonin and Modeller/MOE will be presented.

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An Efficient Monte Carlo Algorithm for Simulating Michaelis-Menten Enzyme Kinetics

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Many reactions in biochemical networks involve enzymatic catalysis, and can be described by standard Michaelis-Menten kinetics. Numerical simulation of the underlying mechanism can be inefficient with either continuous or Monte Carlo algorithms, because of unmatched timescales (stiffness) of the rates involved. Often, the rate of enzyme-substrate dissociation is much higher than the rate of product creation. In this study, removal of the explicitly simulated reversible intermediate was investigated, in order to reduce stiffness and allow for longer timesteps. Reaction rates were adjusted to compensate for lack of the reversible step. Monte Carlo simulations of traditional Michaelis-Menten kinetics were compared to simulations using the new algorithm over a range of timesteps. The results demonstrate the correctness and increased simulation efficiency of the new algorithm, which ultimately may be incorporated into simulation programs such as MCell.

79

The Effect of Non-uniform Acetylcholine Receptor Distribution in Neuromuscular Junctional Folds

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Neurotransmission is the main communicative process in the body, making it an interesting and important area of research. In the vertebrate neuromuscular junction (NMJ), miniature endplate currents (mEPCs) are produced when acetylcholine binds to postsynaptic receptor channels (AChRs) leading to channel opening. Minis exhibit a first-order exponential decay when acetylcholinesterase is inhibited; we hypothesized that this results from a combination of tortuous diffusion space and non-uniform postjunctional fold AChR distribution. This hypothesis was then tested using a large scale 3-D reconstruction and MCell simulations. A preexisting model of the NMJ with constant AChR densities was modified; the top of the folds were populated with AChRs at 7-10,000 μm^2 and the bottom was left unpopulated. Minis were simulated, and the shape of the decay phase and the variability in mEPC decay time were analyzed. Consistent with our hypothesis, an increase in first order decay phase characteristics were seen.

80

The Role of Backbone Flexibility in Protein-Protein Docking

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Protein-protein docking is one of the great modern challenges for computational biology. The Critical Assessment of Protein Interactions (CAPRI), hosted by the European Bioinformatics Institute, has encouraged the community to develop methods for predicting these interactions. Current methods rely on the assumption that a protein is a rigid body. These algorithms do not perform well, especially if the protein undergoes induced fit upon binding. We propose that the use of the Gaussian Network Model can systematically identify regions of flexibility that play a role in induced fit. We shall attempt to show that the Anisotropic Network Model can generate conformations that can be used as rigid bodies in the standard protein docking protocols.

81**Computational Quantitative Structure-Activity Relationship Analyses and Docking to Tubulin of Discodermolide, Dictyostatin-1 and Synthetic Analogues**

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The stabilization of microtubules leads to cell apoptosis. Therefore, microtubule stabilizers make good anticancer agents. Computational molecular models of the microtubule stabilizers dictyostatin-1, discodermolide and synthetic analogues of each were built with the Cerius 2 molecular modeling suite and analyzed for low energy conformers with the Merck Molecular Force Field. A receptor model was then generated using the superimposed structures weighted by their respective biological activities. Grid point interaction energies were calculated from the receptor model. Additional shape, electronic, and thermodynamic descriptors were calculated from the models, and the genetic function approximation was used to generate quantitative structure-activity relationship equations. The GOLD algorithm was used to dock the models to a model of the α -tubulin heterodimer built from coordinates determined by high-resolution cryoelectron microscopy. Energetics of the different orientations within the binding site were calculated. Docking statistics were used with biological activity values in order to form a quantitative relationship.

82**Characterization of Human APE/Ref-1 Protein Family**

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The human APE/Ref-1 protein family is important for DNA repair and regulation of gene transcription. 124 sequences were identified as belonging to family using the superfamily annotation of the iProClass protein database. The sequences ranged from 300 – 600 amino acids in length. An initial multiple sequence alignment was created with the T-COFFEE program and refined using the results of a MEME analysis on the unaligned sequences. This refined alignment serves as the starting point for an extensive analysis of the enzyme superfamily as well as the organization of and differences among its subfamilies. A phylogenetic analysis and a SeqSpace analysis followed to define these subfamilies and their members' sequences. The subfamilies were analyzed for their distinctive features. These features were then mapped onto a 3D structure to see how the structures differ amongst subfamilies.

83**Exploring Sub-optimal Sequence Alignments and Scoring Functions for Comparative Protein Structural Modeling**

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As structural genomics initiatives gain momentum within the structure

determination community, comparative modeling of protein structures will grow both more common and more useful in informing further experimental design. A major challenge in this model construction remains the sequence alignment between the target sequence to be modeled and the template sequence upon which the model will be based. We performed comparative modeling based on sub-optimal sequence alignments between CASP5 target sequences and structural templates of 15-30% sequence identity. Ensembles of 100-500 models per target were produced and scored using a statistical potential function, after which poorly aligned loop regions of the best-scoring model were refined using *ab initio* protein folding simulations. We find that models produced through our procedure are often more accurate than those constructed from a T-coffee alignment, although our scoring process appears unable to identify the absolute best model from the ensemble.

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Application of Hydrogen Bond Analysis Techniques to Protein-Sugar Interactions

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Hydrogen bonding is an electrostatic interaction that is responsible for a wide variety of molecular properties. Hydrogen bonding is especially important in protein chemistry, as these interactions play a pivotal role in the determination of protein structure and binding specificity. The objective of this research project is to study the binding of b (1 à 4)-linked *N*-acetyl glucosamine (GlcNAc) homopolysaccharides to a chitinase-like lectin, human cartilage glycoprotein (HCGP39), a protein involved in connective tissue remodeling processes which is often overexpressed in certain types of breast and colon cancer. Through molecular docking and subsequent geometric hydrogen bond analysis and binding studies, in sight into the binding mechanism of HCGP39 and other Family 18 chitinases will be gained, and these results will be presented and discussed.

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Heterogeneity in Acetylcholine Receptor Kinetics in MCell Simulations: Achieving Known Variability in mEPC Decay

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Vesicular release of acetylcholine (ACh) at the neuromuscular junction (NMJ) produces miniature endplate currents (mEPCs) as ACh binds to ACh receptors (AChRs). In previous MCell (Monte Carlo simulation program) simulations the observed variability of mEPC decay times was much less than experimental results, despite use of detailed cleft topology and acetylcholinesterase distribution. Thus, we hypothesize that different gating kinetics from one AChR to another may explain the experimental variability. The rates for AChR opening and closing were varied based on the known range of mEPC decay times, and then the fractional amounts of the different AChRs were varied in the postsynaptic membrane according to a Gaussian distribution. Under these conditions, simulations still could not reproduce the experimental variability in mEPC decay time, suggesting that spatial segregation of AChRs with different gating properties may also be required.

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Studies of the Hydrosilation Reactions of Diallyltrifluoroacetamide with Alkyl and Fluoroalkyl Silanes

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Studies were performed on the hydrosilation reactions of *N,N*-diallyl-2,2,2-trifluoroacetamide with chloroplatinic acid using various highly fluorinated silanes to improve the yield of the desired *bis*-[*tris* -(perfluoroalkylethyl)silyl

propyl] trifluoroacetamides. Model studies were undertaken with non-fluorous silanes to optimize reaction conditions. The results of these studies were then applied to produce compounds of the general structure $(R_3Si(CH_2)_3)_2NCOF_3$ in which $R = CH_2CH_2C_6F_{13}$ or $CH_2CH_2C_8F_{17}$. The compounds were analyzed by GC, NMR and EI-MS. These compounds have applications as effective electron spray mass spectroscopy calibration standards. Attributes include a large mass range (100-3000 Da), evenly and regularly spaced fragments, and volatility.

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New Free-Amine Polymers and Uses in Tissue Engineering

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Although the use of biodegradable polymers such as Poly(caprolactone), Poly(lactic acid-co-glycolic acid) and Poly(lactic acid-co-lysine) in tissue engineering is not a new concept, their effectiveness is limited. As an alternative approach, the synthesis of a biodegradable, free amine-containing polymer to which essential cell-growth factors can be attached, has come to the forefront. Two similar polymers, identical with the exception of their respective protecting groups, tert-butoxycarbonyl and carbobenzyloxy, are studied within. Following the addition of the amine-protecting group, both compounds undergo the same reaction process through to polymerization. These similar polymers can then be deprotected to yield the identical free amine-containing polymer. This polymer may be studied for future use with the incorporation of amino acid sequences known to target specific cellular receptors associated with cell growth and tissue formation.

Abstracts will become available the week of July 27th

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