441-Pos Board B320
The First Total Synthesis Of Morusin And Himanimide D As Arachidonate 5-lipoxygenase Inhibitor In Automated Docking
Yean-Jang Lee[1], Chia-Fu Chang[1], Cheng-Wei Lin[1], Yu-Chao Huang[1], Chao-Chin Hu[2], Yen-Min Tsheng[2], Tsui-Hwa Tseng[2].
[1]Changhua University of Education, Changhua, Taiwan, [2]School of Applied Chemistry, Chung Shan Medical University, Taichung, Taiwan.
Our research group has exhibited a long-standing interest in the synthesis and bioassay study of a number of unique natural products containing flavonoids, camphorataimides, and benzofurans. In previous works, we have shown that tumor suppressor protein p53 and p38 MAPK (mitogen activated protein kinase) play a prominent role in the caffaic acid phenethyl ester (CAPE) induced-apoptosis of C6 glioma cells. In addition, studies on the constituents of the Chinese crude drug ''Sang-bai-pi'' (*Morus* root bark) have been recorded in books of traditional Chinese herbal medicines, which are used as an antiphlogistic, a diuretic, an expectorant, and anti-hepatitis B activity. Herein, we present the first total synthesis of morusin and himanimide D, and then show the development of the COX/LOX inhibitors by an automated docking method. Morusin, which can be isolated from Chinese herbal medicine, is achieved in which the longest linear sequence is only 13 steps in 12% overall yield from commercially available phloroglucinol. In addition, to probe accessibility of the active sites of proteins, particular COXs/LOXs, the binding affinity of protein-ligand complexes were examined by an automated docking method. Consequently, the rationally optimized LEE-BNOHDM was identified as a potent inhibitor of the arachidonate 5-lipoxygenase.

442-Pos Board B321
Simultaneously Targeting Multiple Drug-Resistant Variants with Optimally Small Drug Cocktails: Application and Analysis of Novel Methods
Andrea P. Johnston, Mala L. Radhakrishnan.
Wellesley College, Wellesley, MA, USA.
Target mutation is a key problem in drug design. High mutational variation of a drug target leads to resistance: drugs which may have originally functioned as inhibitors lose potency because structural changes in the target lead to losses in binding affinity. One way to approach this problem is through the use of drug cocktails. Using integer-programming and physics-based energy functions, we can select an optimally small cocktail from the combinatorial space of possible drugs built from molecular fragments. This computational framework has been presented in previous work by Radhakrishnan and Tidor (*J. Chem. Inf. Model.*, 2008). Current investigations involve analyzing and improving the efficiency of this method and applying it to rapidly varying targets in the HIV-1 system.

443-Pos Board B322
High Throughput In-silico Screening Against Flexible Protein Receptors
Horacio E. Sánchez.
Forschungszentrum Karlsruhe, ggenstein-Leopoldshafen, Germany.
While modern docking methods often predict accurate binding modes, affinity calculations remain challenging and enrichment rates of insilico screening methods unsatisfactory. Inadequate treatment of induced fit effects is one major shortcoming of existing in-silico screening methods. Here we investigate enrichment rates of rigid, soft and flexible receptor models for 12 diverse receptors using libraries containing up to 13000 molecules. For the rigid-receptor model we observed high enrichment (EF_1>20) only for four target proteins. A soft receptor model showed improved docking rates at the expense of reduced enrichment rates. A flexible sidechain model with flexible dihedral angles for up to 12 aminoacids increased both binding propensity and enrichment rates: EF_1 values increased by 35% on average with respect to rigid-docking (3-8 flexible sidechains). We find on average 4 known ligands in the top 10 molecules in the rank-ordered databases for the receptors investigated.

Protein Aggregates I

444-Pos Board B323
Structural Investigation of an SH3 Amyloid Protein Fibril
Marvin J. Bayro[1], Thorsten Maly[1], Neil R. Birkett[2], Christopher M. Dobson[2], Robert G. Griffin[1].
[1]Massachusetts Institute of Technology, Cambridge, MA, USA, [2]University of Cambridge, Cambridge, United Kingdom.
The conversion of soluble proteins into amyloid fibrils and the structural characterization of these aggregates are outstanding problems in molecular biophysics. The amyloid-forming properties of the SH3 domain of the p85a subunit of phosphatidyl-inositol-3-kinase (PI3-SH3), an 86-residue protein, have been extensively studied in vitro by molecular biology methods. However the structure of the protein in fibril form remains unknown, and the specific structural interactions that drive its quaternary arrangement into fibrils have not been elucidated in detail. Since fibrils are insoluble and yield limited x-ray diffraction data, solid-sate NMR spectroscopy is the best-suited method with which to obtain high-resolution structural information of these systems. We present the results of solid-state NMR experiments aimed at elucidating the structure of PI3-SH3 in amyloid fibril form. The spectra of uniformly labeled PI3-SH3 present generally narrow 13C and 15N linewidths, which is characteristic of a high degree of microscopic order and structural homogeneity. Furthermore, samples grown using [2-13C] glycerol as the sole carbon source simplify the spectra and facilitate the observation of long-range internuclear correlations. Employing these alternating labeled samples and a simple data collection protocol consisting of two-dimensional homonuclear and heteronuclear correlation experiments, we have assigned ~75% of the resonances, with the majority of unassigned residues being absent from dipolar correlation spectra due to dynamic or static disorder. Chemical shift analysis is then used to predict secondary structure elements in the PI3-SH3 sequence. The structural characteristics of PI3-SH3 fibrils revealed by solid-state NMR are described in relation to known structural and mechanistic data from previous PI3-SH3 studies.

445-Pos Board B324
Solid-State NMR Studies of the Fibril Forming Protein PABPN1
Daniel Huster[1], Holger A. Scheidt[1], Grit Lodderstedt[2], Elisabeth Schwarz[2].
[1]University of Leipzig, Leipzig, Germany, [2]Martin Luther University, Halle, Germany.
An N-terminal extension of the alanine-rich stretch of the nuclear poly(A) binding protein PABPN1 leads to the disease oculopharyngeal muscular dystrophy. Histochemically, the disease is characterized by intranuclear inclusions of amyloid-like fibrils. For the study of the structure and dynamics of PABPN1 by solid-state NMR a N-(+7)Ala variant of PABPN1(1-132) was uniformly ^{13}C and ^{15}N labelled. Using different NMR techniques (direct, cross polarization, INEPT transfer), variations in the protein dynamics were revealed. Especially the Ala signals show a largely increased intensity in cross-polarized (CP) spectra indicating the presence of a rigid structure for the poly-alanine segment. Most amino acid types s of PABPN1(1-132) are in a random coil conformation. In contrast, for the alanine residues of the protein it was possible to distinguish between two different conformations: A β-sheet conformation was preferentially observed in CP excited spectra and a random coil structure in directly excited or INEPT spectra. Therefore, we conclude that poly-alanine segment of PABPN1 adopts a β-sheet conformation. Additionally, a magnetization transfer form Ala to Gly, which are located next to the alanine stretch, was observed in 2D proton-driven spin diffusion experiments. This indicates that the fibril is extended over the entire poly-alanine segment. To investigate the site-specific dynamics along the backbone of PABPN1(1-132) the CH order parameters for the Cα atoms were measured in DIPSHIFT experiments While the non-fibrillar part of the proteins exhibit low order parameters and has to be considered as quite flexible, the order parameter for the Ala stretch including neighbouring Gly residues are largely increased and characterize this domain as rather rigid. We conclude that the poly-alanine segment forms a β-sheet conformation and forms rigid fibrils, while the rest of PABPN1(1-132) adopts a highly mobile and flexible random coil conformation.

446-Pos Board B325
Solid-state NMR Investigation of b2-Microglobulin Fibril Structure and Dynamics
Galia T. Debelouchina[1], Marvin J. Bayro[1], Geoffrey W. Platt[2], Sheena E. Radford[2], Robert G. Griffin[1].
[1]Department of Chemistry and Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA, USA, [2]The Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom.
β_2-Microglobulin (β_2m) is a 99-residue protein with a classical immunoglobulin fold that constitutes the light chain of the MHC-I complex. In long-term hemodialysis patients, the concentration of β_2m in the blood serum is highly elevated and the protein forms amyloid fibrils that typically deposit in osteoarticular tissues. Although there have been numerous studies that have addressed the process of fibril formation and the structure of the fibrils formed by a small fragment of the protein, atomic level structural information on the full-length fibrils is still lacking. Here, we present solid-state NMR data on the long straight fibrils formed by the full-length protein at pH 2.5, including resonance assignments of a significant number of the residues as well as experiments aimed at characterizing the fibril dynamics. The relationship between these findings and the aggregation behavior of β_2m will be discussed.