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Structure-based Drug Design

3255-Pos Structural Insight Into Mechanisms Of Antibody Mediated Inhibition Of EGFR

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Board B558

The epidermal growth factor receptor (EGFR) is aberrantly activated in a variety of epithelial cancers and has been the focus of much interest as a therapeutic target in anti-cancer therapy. Here we characterize the inhibition of EGFR dimerization and activation by an antibody drug that is currently in phase I/II clinical trials. The progress of our structural and biochemical studies is presented. We compare our results with those known for the antibody cetuximab/ Erbitux®, which is FDA approved for colorectal as well as head and neck cancer since 2004. Both antibodies bind to domain III of the receptor, but show different inhibition mechanisms. The similarities and differences of the two antibodies have important implications for the development of new therapeutic approaches of EGFR targeting.

3256-Pos Crystal Structure of the Complex of Cameline Peptidoglycan Recognition Protein with Disaccharide at 3.2Å Resolution

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Board B559

Peptidoglycan Recognition Protein (PGRP) is a soluble, conserved pattern recognition protein of vertebrates and invertebrates that binds to peptidoglycans (PGNs). PGNs form a group of conserved microbial motifs (Pathogen - associated molecular patterns-PAMPs) that are unique products of microbial metabolism not produced by the host. PGNs are located on the surface of virtually all bacteria and fungi and as such, constitute excellent targets to recognition by PGRPs. We have isolated a 20 kDa PGRP from cameline mammary secretions. It has been crystallized in the space group I222 with cell dimensions, a = 89.9Å, b = 102.5 Å, c = 164.2 Å having 32 molecules in the unit cell. The structure reveals the presence of two crystallographically independent dimers unlike human PGRP, which is a monomer. PGN binding groove is located in the domain close to C-terminus. The molecular structure contains a central β-sheet composed of five β-strands, four parallel and one (β5) antiparallel and three α-helices. PGN binding site resides in a long cleft whose walls are formed by helix α1 and five loops β3 - α1, α1- β4, β5- β6, β6 - α2 , β7 - α3. The second site is located on the opposite side of the proteins to the PGN-binding site, which apparently accommodates host effector or signaling molecules. It is formed by variable PGRP-specific segment and helix α2. Disaccaride is observed in the Ligand binding cleft and interacts with residues such as Glu 52, Asn 55 and Thr 38 of the A molecule.

3257-Pos A Computer Modeling Approach towards Designing Dual LOX/ COX Inhibitors as Potent Anti-Cancer drugs

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Board B560

The role of cyclooxygenase2 (COX2) enzyme in cancer promotion has been discovered almost a decade back. But the novel mechanism by which lipoxygenase (LOX) enzyme and its products are co-promoting the COX2 in cancer cell proliferation is now raising a new question. Will the dual inhibition of COX2 and LOX enzymes block the cancerous proliferation of cells and if so to what extent? Preliminary studies done on this novel mechanism of cancer prevention by inhibiting COX2/5LOX have shown excellent positive results.

Keeping this in mind, we have used the available X-ray crystal structures of the complexes of COX2 and LOX with the known inhibitors to carry out a structure-based, rational, molecular modeling approach to design a small peptide inhibitor, which is potent for both COX and LOX. Since the crystal structure of 5LOX is not known, and since the active sites of human 5LOX and mammalian 15LOX are highly similar, the crystal structure of rabbit 15LOX enzyme has been used. Docking studies using Discovery Studio 1.7 (Accelrys Software Inc) indicate that the designed peptide inhibits both 15LOX and COX2 with potency in the nanomolar range, which is about 1000 times more than the known dual LOX/COX inhibitors. Furthermore, this designed inhibitor also blocks the COX1 enzyme so that the unwanted cardiovascular side effects of COX2 selective inhibitors are avoided. Thus, the designed small peptide inhibitor is a novel lead compound for the design of a new class of anti-cancer drugs.

3258-Pos Free Energy Calculations on the Binding of Thiolactomycin Derivatives to E. Coli Fatty Acid Synthase I

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Board B561

Finding novel antibiotics to combat the rise of drug resistance in harmful bacteria is of enormous importance for human health. Computational drug design is an emerging field that is concerned with aiding synthetic chemists in this search for new potent inhibitors. In recent years, molecular dynamics based free energy calculations have emerged as a useful tool to accurately calculate binding affinities of novel or modified ligands. While being significantly more demanding in computational resources than simpler docking algorithms, they can be employed to obtain reliable estimates of the effect individual functional groups have on protein-ligand complex binding constants.

In this work, we analyze the effect of several chemical modifications on the binding of the natural inhibitor thiolactomycin to beta-ketoacyl [acyl carrier protein] synthase I from E. coli . KAS I facilitates a critical chain elongation step in the fatty acid synthesis pathway. Since the bacterial type II lipid synthesis system is fundamentally different from the mammalian type I multi-enzyme complex, it represents a promising target for the design of specific antibiotics.

We employed thermodynamic integration calculations to predict the effect of changing functional groups on the thiolactomycin scaffold. The Amber9 molecular dynamics suite, with additonal modification to improve phase space sampling in free energy calculations, was used for calculations. Several ligand modifications were found that could lead to thiolactomycin derivatives with improved binding constants in the nanomolar range. When the six most promising new compounds were synthesized and screened for activity, they strongly inhibited bacterial growth.

3259-Pos MDock: An Automated Molecular Docking Tool for Ligand Binding, Virtual Screening and Selectivity Studies with the Inclusion of Protein Flexibility

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Board B562

MDock is a suite of molecular docking program targeting single or multiple protein structures. Efficient docking of a ligand against multiple protein structures is an extremely useful tool for inclusion of protein flexibility in ligand binding, prediction of ligand specificity, and rapid identification of inhibitors for a family of proteins. The MDock program integrates the sphere-ligand matching algorithm of UCSF DOCK (Ewing and Kuntz, J Comput Chem *18*, 1175–1189, 1997), the **ITScore** energy scoring function (S.-Y. Huang and X. Zou, J Comput Chem *27*, 1866–1875; 1876–1882, 2006), and the **Ensemble Docking** algorithm (S.-Y. Huang and X. Zou, Proteins *67*, 399–421, 2007). MDock can simultaneously dock a ligand into the userdefined binding site of the multiple protein structures with little additional computational demand. The software accounts for protein flexibility in ligand binding by using an ensemble of multiple crystal structures or NMR conformers of the same protein (S.-Y. Huang and X. Zou, Protein Sci *16*, 43-51, 2007). Here, MDock was tested on rapid identification of inhibitors for a family of homologous proteins by simultaneous virtual database screening against an ensemble of 18 protein kinases. The obtained enrichments were shown to be comparable to those from the standard single-target docking protocol for the individual kinase protein. MDock was also applied to study ligand selectivity against different target proteins by testing seven typical protein kinase inhibitors against the above protein kinases. MDock is computationally efficient, and the run time is almost independent of the number of protein structures and on average consumes ~ 2s on a single 3.2 GHz Pentium IV CPU for docking one ligand conformer. MDock can be obtained *free of charge* for academic users.

3260-Pos Targeting the EGF Receptor by Virtual Screening

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Board B563

It is estimated that one in eight American women will develop breast cancer in her lifetime and a significant number of these cases are due to misregulation of EGFR and ErbB2, two members of the epidermal growth factor receptor family (ErbB). In particular, 70–80% of metaplastic breast carcinomas overexpress EGFR, and 30% of all breast carcinomas overexpress ErbB2. Breast tumors overexpressing one or both of these receptors are associated with aggressive clinical malignancy. Current drugs based on large antibodies (mAb) and tyrosine kinase inhibitors (TKIs) show promising yet highly variable clinical efficacy. In addition, serious side-effects like cardiotoxicity and comprised immune system further restricts their clinical effectiveness. Recent advancements in the regulation of ErbB-signaling suggest that these therapeutic limitations are due to the underestimation of a much more complex system - a system that relies on inter-receptor interactions through the process of homo- and hetero- dimerization. These new findings therefore motivate the development of novel inhibitors that target these receptor-receptor interactions. The objective of this project is to identify the first class of small-molecule inhibitors that target and disrupt the dimerization. We are applying a cost- and time- effective protocol that combines in silico virtual high-throughput screen (vHTS) and in vitro experimental assays to identify lead compounds. Our approach contrasts the traditional strategies of developing mAb- and TKI-based inhibitors. The application of computational methods provides a realistic way to screen and test thousands of compounds. We have experimentally tested top compounds predicted by the vHTS and

have identified several candidate inhibitors with promising potency and specificity in living cells. Our findings can serve as a building block that carries promises for clinical benefit in treating ErbB-driven breast cancers.

3261-Pos Optimization of Inhibitors of the Human Cytoplasmic Protein Tyrosine Phosphatase

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Board B564

Inhibition of the human cytoplasmic protein tyrosine phosphatase (HCPTP) provides a mechanism by which to down regulate the metastatic phenotype present in human epithelial tumors caused by the underphosphorylation of the EphA2 receptor tyrosine kinase. Inhibitors for each isoform of HCPTP have been identified by *in silico* screening of small molecule libraries as well as through rational design. *In vitro* kinetic analysis has verified the discovery of low micro-molar inhibitors of HCPTP, which are the most potent inhibitors of HCPTP reported to date. Common structural elements have been identified among these strong inhibitors that agree with rationally designed elements. Structural information of inhibitors bound to the enzyme is now necessary. Crystal structures of both isoforms of HCPTP have been generated within the laboratory and crystallographic analysis of inhibitor-enzyme complexes can now proceed. Additional enzymatic analysis of mutants of HCPTP will be performed in order to elucidate the roles of individual amino acids that line the entrance of the active site of each isoform of HCPTP in the binding to inhibitors. Comparison of efficiency of inhibitors at several pHs will aid in the elucidation of physiological roles for each isoform of HCPTP. Each of these results will direct inhibitor development as well as elucidate the future of isoform-specific inhibitors of HCPTP.

3262-Pos Physicochemical studies on Lanreotide self-association process in water

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Board B565

Self-assembly of peptides is a recurring theme in research and one of the most important strategies used in wide range of applications in biotechnology and material sciences. Indeed, a better understanding and control of the hierarchical structure formation should drive us to a better knowledge of biological processes in general. This in turn will lead us to new conceptions of biomimetic nanomaterials.

We are working on Lanreotide, a synthetic analogue of the natural Somatostatin. The lanreotide, an octapeptide, self-assembles into well defined hierarchical structures. Currently, Lanreotide acetate is used as a therapeutic peptide in the treatment of acromegaly in the form of a gel at high concentrations (3–30%w/v). The self-assembling into nano-tubes of Lanreotide acetate in water strongly suggests a correlation between Lanreotide nano-organization and the controlled release properties of this pharmaceutical product during medical treatment. Here we chose a multidisciplinary approach to study lanreotide and its derivatives, by combining polarized light microscopy, electron microscopy, vibrational spectroscopies, small and wide angle X-ray scattering, and analytical ultra centrifugation to elucidate:

(i) -the hierarchical structures formed and
(ii) -the molecular and supramolecular self-assembly mechanism.

We have recently shown the self-assembly occurs through the association of β-sheets driven by amphiphilicity and a systematic aromatic/aliphatic side chain segregation. The assembly is the result of a fine equilibrium between:

(i) -ionic strength,
(ii) -hydrophobic effect and
(iii) -H-bond network.

As a result we are able to govern the morphology of the object formed: fibres, ribbons and nanotubes with specific diameter. This original and simple system is a unique example of molecules able to self-organize into a well-defined nanostucture. The supramolecular organization of Lanreotide and derivatives demonstrates that this system is able to investigate the minimal interactions required for generating large self-assembling nanoscale structures, including proteins and could find implications for β-amyloid fibers.

3263-Pos Complete Binding Parameter Determination with 200 vl of Sample

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Board B566

Modern, highly sensitive Isothermal Titration Calorimetry (ITC) instruments are being applied in the drug discovery and development process for applications such as selection of small molecule "hits" following primary and secondary screening of chemical libraries against protein targets of interest, optimization of small molecule leads based on elucidation of the binding mechanism and binding characteristics and development of structure-activity-relationships (SAR) that are used in the optimization process, and selection and optimization of therapeutic protein variants. As modern ITC instrumentation has evolved, these instruments have become more sensitive, faster and easier to use. Binding parameters determined by ITC are often referred to as the "gold standard" values and are frequently used as reference standard values for other techniques. Despite the fundamental advantages of this technique and the advances in instrumentation, use of ITC in the early, critical decision-making stages of drug discovery is often limited due to the

lack of sufficient quantities of protein during those early stages and the time required to perform the ITC experiments on large numbers of potential ligands (or protein constructs) of interest. Traditionally 50 -1500 μg of protein has been consumed to complete an ITC experiment and completing each experiment could require two hours or more. Data presented we will describe the characteristics of a new miniaturized, ultrasensitive ITC that has been designed to push back these limitations allowing ITC to be effectively utilized at earlier stages of the drug discovery and development process.

3264-Pos Structure-Activity Relationship Between Lipopolysaccharide And An Antimicrobial Fragment Of Human Cathelicidine

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Board B567

Human cathelicidine hCAP18 is a potential lead structure for new antibiotics to overcome the rising number of pathogenic multidrug resistant bacterial strains.

In the case of Gram-negative bacteria, the first contact sites of antimicrobial peptides are the molecules of the outer leaflet of the outer membrane, mainly Lipopolysaccharide (LPS). LPS is able to induce an inflammatory response, which may cause the fatal septic shock.

To investigate the structure-activity relationship between LL-32, an active fragment of hCAP18, and LPS, especially the influence of the core oligosaccharides, we used fragments of LL-32, lacking some amino acids residues, and *Salmonella enterica* serovar Minnesota strains differing in the length and charge of the core oligosaccharides. *In vivo* hCAP18 is processed to even shorter peptides than LL-32, so these fragments might play an important role in the immune system.

We determined the antibacterial and antiinflammatory activities of these peptides against the *S. enterica* strains. Furthermore, we measure the interaction of the fragments with membrane-models composed of different LPS with physical methods. Binding of LL-32 to LPS and bacteria was investigated by ITC and by measuring the Zeta-potential. After binding to LPS, LL-32 induce lesions or disrupt the membrane by micellisation. We measured the ability of the fragments to fuse LPS-aggregates using a spectroscopic assay based on Förster Resonance Energy Transfer. Furthermore, we determined the size of LPS-aggregates in dependence of the LL-32 concentration. FACS measurements using fluorescently-labelled LL-32 on bacteria showed that LL-32 bind instantly to bacteria leading to a permeabilisation of the membranes.

Our data allows to explain the different biological activities of LL-32 by means of biophysical data. Furthermore, the different behaviour of the used fragments allows identifying specific residues in the AA sequence of LL-32, which are important for its activities.

3265-Pos Dual Mechanism Of Bacterial Lethality For A Cationic Sequencerandom Copolymer Mimicking Host-defense Antimicrobial Peptides

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Board B568

Flexible sequence-random polymers containing cationic and lipophilic subunits, which can be induced by a bacterial membrane surface to adopt globally amphiphilic irregular structures, have been synthesized (Mowery, B.P., *et al.*, submitted for publication). These polymers are mimics of antimicrobial host-defence peptides, which act by disrupting bacterial membranes. We have studied one such copolymer, BPM2-39B, having an average length of 21 residues, which is very active against both Gram positive and Gram negative bacteria, with regard to its ability to disrupt model and bacterial membranes. Our findings show that at very low concentrations, comparable to their MIC values, it is able to permeabilize, in a highly cooperative fashion, model membranes mimicking the lipid composition of *E. coli, S. aureus* and *B. subtilis*. It is ineffective against zwitterionic membranes, which explains its low hemolytic capacity. Both DSC and ITC indicate that it is capable of binding as well as segregating anionic lipids, forming anionic lipid-rich and anionic lipid-poor domains. Experiments with the *E. coli* mutant ML-35p, indicated that permeabilization in Gram negative bacteria is biphasic; at low concentrations (up to 25 μg/mL) the polymer is capable of permeabilizing the outer membrane, which is blocked at higher concentrations of polymer. Experiments with *E. coli* K-12, showed that at very low concentrations the polymer is also able to reach and disrupt the inner cytoplasmic membrane. Despite the fact that at higher concentrations the polymer does not permeate the inner membrane, it associates with the negatively charged LPS layer (or LTA in gram positive bacteria), with lethal consequences for the organism. We propose then a dual mechanism of bacterial killing by flexible sequence-random copolymers, which differ at low and high concentrations of polymer.

Regulatory Networks & Systems Biology - I

3266-Pos Closing The Loop: Towards A Comprehensive View Of Action At A Distance In Transcriptional Regulation

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Board B569

DNA architecture plays a key role in determining spatial and temporal patterns of gene expression. This architecture encompasses both the nucleotide sequence (i.e., the information content)