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Protein Structure-Based Drug Design Session and Related Posters (Chair: Christophe Verlinde)

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Christophe Verlinde (U. Washington) began this exciting, diverse session with a crisp overview of the state of structure-guided drug design. He marked the major challenges facing drug design as pre-assessing drug toxicity, flexibility, and binding energy. The comprehensive overview covered many currently used drug scaffold libraries as well as computer methods for matching pharmacophores and building, linking, and docking drug candidates. Verlinde's results focused on inhibitors developed to halt potentially lethal trypanosomiasis (African sleeping sickness) by blocking the cofactor site of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Targeting the cofactor rather than active site came from the bright observation that human and trypanosomal GAPDH active sites were highly conserved, but the cofactor site differed enough to allow a parasite-specific inhibitor. A 45-fold selective inhibitor was achieved by modifying substituents on the cofactor's adenosine ring, and novel inhibitors identified by DOCK shape-template searches of the Available Chemicals Database (ACD).

X. Chen (UCSF) presented another important application of DOCK: identifying non-peptidyl drugs for malaria, which kills a million people each year. The malarial protease falcipain was modelled based on papain's structure and a loop dictionary, and its active site used as a DOCK template for searching the ACD. Exhaustive molecular graphics screening led to identification of an oxalic hydrazide with 10 micromolar inhibition. QSAR and structure-guided redesign led to 150 nanomolar inhibitors of even chloroquine-resistant malarial strains. It's exciting that homology modelling can provide a good enough template for successful inhibitor design, and the results show the benefits of attacking a hard problem from all sides. For the loop modelling problem, a poster by Sucha Sudarsanam (Immunex) impressively simplified the task by constructing loops using a dipeptide database. He demonstrated that choosing dipeptide ϕ, ψ values consistent with adjacent residues' preferences and selecting loops with ends overlapping the known structure leaves only a few loop conformations, which are typically close to the correct one.

Next, Trevor Hart (U. Alberta) took us for a non-random walk through ligand docking using BOXSEARCH. A Monte Carlo/simulated annealing algorithm testing a number of random initial ligand positions, BOXSEARCH has a novel way of resolving steric clashes. Its "floating" algorithm uses Boltzmann probability to accept or reject steric overlaps based on overlap energy. Genetic algorithms are being explored to increase searching efficiency.

The structural basis for antibiotic specificity and resistance were the theme of Osnat Herzberg's (CARB) talk. Mutants and structures for class A and C beta-lactamases, which have similar structure but

different specificity (penicillin vs. cephalosporin), imply that the two classes have different mechanisms for beta-lactam cleavage. Mutating just two residues allowed hydrolysis of previously resistant 3rd-generation cephalosporins, a sobering result. Natalie Strynadka's (U. Alberta) poster presented the structure of a class A beta-lactamase in complex with BLIP (beta-lactamase inhibitory protein). This mysterious protein binds a spectrum of beta-lactamases with very high affinity, producing an enormous protein:protein interface including BLIP phenyl and carboxyl groups mimicking those in penicillin G. A surprising case of a protein mimicking a previously-known small molecule inhibitor! 24 interfacial water molecules mediate binding and may provide plasticity for binding different beta-lactamases. To win against bacteria and viruses, it's clear that we need to stay one step ahead of their mutations. Insightful strategies for beating drug resistance in HIV protease (HIVP) were presented in Paul Ala's poster (DuPont Merck). While it seems natural to use symmetric inhibitors for an active site formed by two identical subunits, there is a definite advantage to asymmetry: then active-site mutations can only break interactions with half the inhibitor. Developing larger inhibitors can also increase binding and reduce the effect of any single protein mutation.

Pat Weber (Schering Plough) used combinatorial libraries to discover L- and D-peptidyl ligands for streptavidin as reversibly-binding alternatives to biotin. For biotechnology applications, such as tagging and affinity purification, controlling binding affinity can be much more powerful than having a very tight binder (the usual goal of drug design). Scott Dixon (SmithKline Beecham) then presented a computational analog of combinatorial libraries by using a genetic algorithm to "cross over" and evaluate new combinations of 2-dimensional molecular structures as ligand candidates, with penalties being incorporated to disfavor hard-to-synthesize compounds. Using nonbonded interactions as an evaluator, flexible ligands were docked in the protein active site making the assumption that active-site bound water molecules were not displaced. This brought to mind Michael Raymer's earlier genetic algorithms talk, which demonstrated that ligand-displaced and ligand-mediating water molecules can be predicted and appropriately included. Dixon is also analyzing the relationship between binding affinity and structural characteristics such as buried surface area, solvation, and mobility for 240 protein complexes.

Charlie Bugg (Biocryst) gave an inspiring overview of 15 years of pioneering research on purine nucleotide phosphorylase, which is required for T-cell immunity. It was revealing that the crystal structures not only gave insight in how to improve the affinity of leads, but also indicated which parts of the structure should not be touched for improving important physicochemical properties such as solubility. Starting from leads in the milli- to micromolar range, several nanomolar inhibitors were obtained. Importantly, a number of these compounds are in Phase I and II clinical trials for T-cell mediated diseases. This great session was closed by Richard Schevitz (Eli Lilly), who described the iterative design of a selective non-pancreatic phospholipase A2 inhibitor. A take-home message from Richard's talk was that examining the structure and using common sense are the key to success. After all, it looks as if it's difficult for computer programs to beat the human brain! (And genetic algorithms and combinatorial libraries remind us that combining two brains is better than one.)