The FightAIDS@Home Project uses the volunteered computing power of the World Community Grid to test candidate compounds against the variations (or “mutants”) of HIV that can arise and cause drug resistance. FightAIDS@Home recently identified several fragments as new candidates for a novel binding site on the peripheral surface of HIV protease. These fragments docked well against the “exo site,” and our collaborators recently began in vitro studies (i.e., “wet lab” experiments in test tubes) to assess their potencies. If these wet lab experiments produce promising results, then these fragments could form the foundation for the development of “allosteric inhibitors” of HIV protease (i.e., “flexibility wedges” that can disrupt the conformational changes that HIV protease must undergo in order to function). These allosteric inhibitors could represent a totally new class of anti-AIDS compounds.

To learn more about the process we use to discover, evaluate, and develop potential new inhibitors of HIV protease, see Volume 7 of the FightAIDS@Home newsletter.

FightAIDS@Home results were included in a recent publication
The research manuscript, “AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility,” by G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, and A.J. Olson was recently published in the Journal of Computational Chemistry (volume 30, number 16, pages 2785-2791). In Experiment 24 you and fellow crunchers helped perform “cross-docking” calculations that assisted in the evaluation and optimization of the new AutoDock code and the new scoring function that it uses to evaluate the strength of ligand—protein interactions. These cross-docking calculations, which were performed on the WCG during August of 2008, included docking all of the known HIV protease inhibitors against 100 different crystal structures of HIV protease. Thank you very much for your assistance with the calculations used to produce these results.
Follow-up on Experiment 22: the best-ranked compounds from the “DTP library” failed the second round of “wet lab” experiments

In Experiment 22, which you helped us perform on the WCG from October 2008 to March 2009, the NCI’s “DTP library of moderately active compounds” was screened against the “exo site” on the sides of HIV protease. From the results of this experiment on FightAIDS@Home, we identified 12 compounds as potential allostERIC inhibitors. We were able to obtain samples of 7 of these 12 compounds from the NCI. Our collaborator Dr. Y.C. Lin (in Prof. John Elder’s lab at The Scripps Research Institute) evaluated these compounds in the standard “FRET-based” anti-HIV protease activity assay. At low micro-Molar concentrations, 4 of these 7 compounds displayed anti-HIV PR activity against both the wild type and the V82F/I84V multi-drug-resistant mutant “super bug”. The two best compounds, NSC11243 and NSC16224, displayed IC$_{50}$ values of 4.83 ± 0.54 and 0.89 ± 0.09 micro-Molar, respectively, against the wild type protease. The IC$_{50}$ value indicates the concentration of an inhibitor that decreases an enzyme’s activity by 50%. The compound NSC11243 is shown below as sticks with green carbon atoms, docked against the surface of a representative conformation of the V82F/I84V multi-drug-resistant mutant, which was harvested from Molecular Dynamics simulations published in Protein Science in 2004 by A.L. Perryman, J.H. Lin, and J.A. McCammon. NSC16224 is shown below on the right as sticks with magenta carbon atoms, docked against a different representative conformation of that mutant “super bug.”

Although our docking results and the first round of wet lab experiments both seemed very encouraging, subsequent assays performed with the presence of small amounts of a detergent (Triton X-100) indicated that these compounds are non-specific inhibitors (i.e., promiscuous binders). Compounds that non-specifically inhibit many different macromolecules are likely to cause serious toxic side effects. Consequently, we will not pursue the development of these compounds from the DTP library. In addition, we decided to start focusing our efforts on completely different libraries of compounds.
New approach: Fragment-based virtual screens vs. the exo site yield promising results

We recently finished analyzing the results of Experiment 27, which you helped us perform on the World Community Grid during March and April of 2009. This experiment also searches for “flexibility wedges” that bind to the peripheral surface of HIV protease, but in this experiment we used a different library of compounds, and we docked these compounds against a new fragment-induced crystal structure of HIV protease, which our collaborator, Prof. C. David Stout, recently produced. In these docking calculations, the ChemBridge library of approximately 12,000 different building blocks (i.e., compounds with a size corresponding to one or two fragments) was screened against the exo site of wild type HIV protease. In wet lab experiments at The Scripps Research Institute, our collaborators will test the best-ranked compounds harvested from these AutoDock calculations. They will evaluate these fragments against both wild type and multi-drug-resistant variants of HIV protease.

You might wonder why we now use “fragment-based” virtual screens on FightAIDS@Home. After the surge of new high-throughput technologies for drug discovery that emerged in the 1990’s, the focus has shifted from the volume of hits discovered to their quality. A crucial measure of hit quality is “ligand efficiency,” which is defined as the free energy of binding divided by the number of non-hydrogen atoms in the ligand. The use of fragment-based techniques in drug discovery is founded on the medicinal chemists’ realization that “hits” (see Volume 7 of our newsletter) have to be extended to create new, larger compounds, in order to achieve both high affinity and high specificity with the target. Fragments can be readily extended during this “hit-to-lead” optimization process, but libraries of “lead-like” compounds tend to contain ligands that are already large, complex, and, thus, less extendable. Fragment-based approaches have already been used to develop nano-Molar inhibitors of many different types of drug targets, such as PDE4, β-secretase, Anthrax lethal factor, thymidylate synthase, HPV E2, MMP3, FKBP, JNK1, JNK3, CDK2, and CXCR2. Typically, the hit rate from fragment-based screens is much higher than the hit rates observed with traditional High-Throughput Screens, at least in part due to the fact that libraries of small fragments tend to cover a larger proportion of “low molecular weight chemical space” than the amount of “higher molecular weight chemical space” that is covered by libraries of lead-like compounds. Since we wish to sample as much chemical space as possible in these virtual screens, we are now focusing on these fragment-based approaches.

From the results of this virtual High-Throughput Screen (vHTS) on FightAIDS@Home, we identified the top 113 fragments, which all displayed cluster sizes greater than 70 (out of 100 to 150 runs) and estimated Free Energy of Binding values less than –6.949 kcal/mol. We request and always receive at least 100 independent runs per ligand—target docking job, but due to the redundant distribution of these jobs to your volunteered host machines, we sometimes receive up to 150 runs/job. The top 113 fragments identified contained at least 50% of the runs in the “best energy cluster,” which means that these clusters correspond to both the best energy cluster and to the largest cluster per docking job. In the computer-aided drug discovery community, it is currently unknown whether it is generally more accurate to utilize the results in the best energy cluster or the largest cluster. For some targets the former seems more
accurate, while the latter approach provides better correlations to experimental data with other targets. To get around this tricky issue, we developed new protocols that allow us to fish out compounds that meet both criteria simultaneously.

The binding modes displayed by these 113 fragments were visually inspected by ALP with the new version of AutoDockTools discussed on page 1 of this newsletter. This virtual screen identified 60 potentially useful building blocks, which were all added to our growing “palette of molecular legos”. We are most interested in the compounds that displayed the largest cluster sizes and the highest ligand efficiencies in these AutoDock calculations. Thus far, 13 of the top 46 fragments from this ChemBridge building blocks library were purchased by our collaborator, Prof. C. David Stout. A single gram of each of these 13 fragments cost approximately $1,150.00 from the vendor Hit2Lead. The 46 best-ranked building blocks are shown below as sticks with cyan carbon atoms. As you can see, these building blocks displayed a substantial amount of overlap in their predicted binding modes. The Stout lab will soon start testing these fragments in crystal soaking experiments, and our collaborators in Prof. John Elder’s lab and in Prof. Bruce Torbett’s lab will simultaneously test these building blocks in two different types of HIV protease assays (i.e., the standard FRET-based assay and a new “thermal shift” assay, respectively).

![Image of molecular structures]

**Potential significance of the development of allosteric inhibitors of HIV protease**

By carefully modifying, extending, combining, and characterizing the fragments that bind well to the exo site of the new crystallographic conformation of HIV protease from the Stout lab, we will try to advance the development of larger, higher-affinity compounds that can bind this site and stabilize the conformations of protease that have closed flaps. Because the flaps (a) control access to the active site, and (b) require the ability to open, close, and then open again in order for the catalytic cycle to proceed, these compounds could inhibit HIV protease by allosteric mechanisms. As hypothesized from the results of previous simulations, a large enough
allosteric ligand could restrict compression of residues 38-42 and 59-63, thereby inhibiting the flaps from opening via a completely novel mechanism, due to the anti-correlated motion of these chain segments. Similarly, such ligands could also facilitate closing of the flaps, which means they could potentially help restore the potencies of the current FDA-approved HIV protease drugs against the multi-drug-resistant mutant “super bugs” that are becoming increasingly prevalent in AIDS patients.

All current FDA-approved HIV protease inhibitors target the active site. To improve the long-term treatment of patients infected with HIV, especially those infected by multi-drug-resistant strains, new types of drugs with novel mechanisms of action are urgently needed. In addition to advancing the discovery and development of a new class of inhibitors (i.e., allosteric inhibitors of HIV protease), which could potentially improve the treatment of the strains of HIV that currently circulate in the patient population, these allosteric inhibitors might also have the potential to impede the evolution of new multi-drug-resistant mutant super bugs. If allosteric inhibitors can be developed that stabilize the closed conformation of the flaps, they will probably elicit new protease mutations that counteract these new compounds by stabilizing the open conformations of the flaps. However, if such mutants do arise, they will most likely have decreased catalytic efficiencies, since closed flap conformations are required during the cleavage of the natural viral polypeptide substrate. Thus, the combination of allosteric inhibitors and active site inhibitors might be able to “corner” HIV protease in sequence space, such that it cannot mutate in new ways that allow it to both escape all of the drugs and still perform catalysis efficiently. These ideas represent the foundation of our current virtual screening efforts.

The American Recovery and Reinvestment Act of 2009 (ARRA) provides stimulus for our future FAAH experiments

We would like to share some great news with all of you. Our stimulus-related supplemental funding proposal on drug resistance in HIV integrase got approved! Grant number 3P01GM083658-0251 will enable us to perform virtual High-Throughput Screens against our brand new models of the catalytic core domain of HIV integrase. We’ve developed new protocols that allow us to create more accurate models of this important viral target, against which only one drug has been approved (raltegravir, approved by the FDA in 2007). We then developed new dynamic models of the wild type and of the two most raltegravir-resistant mutants of HIV integrase. These models provide insight regarding the potency of raltegravir against the wild type integrase and suggest why it does not work well against these mutants. We recently submitted a manuscript on these exciting new modeling results, which is currently under peer review. We’ll let you know as soon as it is published. In the near future, we will start performing fragment-based screens on FightAIDS@Home against our new models of drug-resistant HIV integrase.

Assisting the computer-aided drug discovery community

The models of the compounds that we use in these virtual screens were first obtained from the Shoichet lab’s ZINC server at UCSF (http://zinc.docking.org/) as “mol2” files. These files then have to be substantially re-formatted and filtered to enable their use in AutoDock calculations (i.e., they have to be converted into the “pdbqt” format, and compounds with rare atom types
that AutoDock cannot handle properly have to be removed from the library of ligands before the docking calculations are prepared). Due to a few persistent requests from Dr. Alex Perryman and some hard work by Dr. John Irwin in the Shoichet lab, the pdbqt-formatted versions of the libraries of ligands that Dr. Stefano Forli prepared for our experiments on FightAIDS@Home are now being distributed from http://zinc.docking.org/pdbqt. See http://docking.org/?q=node/148 for additional details. The resources that you provide us motivated us to reformat these libraries that contain models of hundreds of thousands of different compounds. Now all of the different labs in the drug discovery community can easily and freely obtain these ready-to-use AutoDock input files for their own virtual screens against drug targets from HIV and from other diseases.

The images displayed in this volume were made with the Python Molecular Viewer (i.e., PMV) by Dr. Alex Perryman.

We could not perform this much research without your help. Thank you very much for helping us advance the fight against multi-drug-resistant “super bugs” of HIV and for helping us improve the tools and techniques that many other labs use in their own research against other diseases.

http://fightaidsathome.scripps.edu