HIV, the virus that causes AIDS, infects over 34 million people, and approximately 2.3 to 2.8 million new people are infected each year. In 2011, 1.7 million people died from AIDS. HIV kills more people than any other virus on Earth. Even if/when we can eventually prevent new HIV infections with vaccines or anti-viral microbicides, we will still need to discover new drugs that can help treat the millions of people who are currently living with an HIV infection. The need to discover new types of drugs against HIV is especially urgent, since new multi-drug-resistant mutant “superbugs” of HIV are constantly evolving and spreading throughout humanity. In addition, other scientists have shown that treating HIV with effective drugs also helps decrease the probability of spreading the infection to new people. When effective drugs are given to a particular patient, the number of infectious viral particles in that patient (or the “viral load”) decreases, which lowers the probability of them infecting other people. It doesn’t eliminate the possibility of spreading the infection, but it does reduce the probability. Consequently, by helping us advance the discovery of new anti-HIV drugs, we have the potential to both assist in the treatment of current HIV patients as well as to help prevent the spread of this deadly virus.

**Why do we perform these calculations?** The FightAIDS@Home Project uses the volunteered computer power of IBM’s World Community Grid to test candidate compounds against the variations (or “mutants”) of HIV that can arise and cause drug resistance. We test these candidates by “docking” flexible models of them against 3-D, atomic-scale models of different drug targets from HIV, to predict (a) how tightly these compounds might be able to bind, (b) where these compounds probably prefer to bind on the protein target, and (c) what specific interactions are likely formed between the candidate and the target. Compounds that can bind tightly to the right regions of particular proteins from HIV have the potential to “gum up” and shut down the viral machinery and, thus, help advance the discovery of new types of drugs to treat HIV infections.

**Summary of recent progress:** FightAIDS@Home calculations led to the discovery of 10 novel fragments that bind HIV protease, shift its stability, and/or affect its conformational preferences. These 10 compounds from Experiment 30 are predicted to bind to either the “4D9 exo site” on the sides of HIV protease or to the potential allosteric site on the top of the flaps of protease (the “1F1 site”). See images A-D on page 2 for the location of these sites. Compounds
that can bind either of these two sites with high affinity (high potency) might eventually lead to the development of completely new types of anti-HIV drugs that can shut down the activity of the active site (the hollow tunnel in the center). We do not yet have any proof about where these compounds actually bind on protease, but our collaborators in the Stout lab are currently trying to generate that evidence. These 10 new fragment hits are predicted to bind to these two potential allosteric sites, and we do have proof that these compounds bind somewhere on HIV protease and affect its stability and conformational preferences. Our collaborators Dr. Max Chang, in Bruce Torbett’s lab, and Dr. Michael Baksh, in M.G. Finn’s lab, have shown that these compounds are able to bind to HIV protease in solution (that is, HIV protease dissolved in water), and they cause dose-dependent effects on its thermal stability (it’s melting temperature, which is the temperature at which the viral enzyme unfolds), and/or its conformational preferences. Importantly, the 4 new hits predicted to bind the 4D9 exo site (the cyan spheres in images A & B on the left) were able to inhibit HIV protease activity in biological assays performed by Dr. Ying-Chuan Lin in John Elder’s lab. Although these 4 fragments are very small, weak inhibitors of HIV protease, they represent the first compounds that we have ever identified in virtual screens against these two sites that were able to inhibit HIV protease activity in wet lab experiments. Once our collaborators demonstrated that these fragments display activity against HIV protease, these four new hits were nicknamed “APex41” to “APex44” (for Alex Perryman’s exo site-4D9 compounds 1-4).

The discovery of these two potential allosteric sites (that we are currently targeting) was presented in the manuscript “Fragment-Based Screen against HIV protease,” by Alexander L. Perryman, Qing Zhang, Holly H. Soutter, Robin Rosenfeld, Duncan E. McRee, Arthur J. Olson, John E. Elder, and C. David Stout, in the journal Chemical Biology & Drug Design, 75(3), pages 257-268 (2010).

Read the rest of this newsletter to learn about the details of this experiment and how we are following up on it. For a general summary of the FightAIDS@Home project, watch the YouTube clip of Dr. Alex Perryman’s interview by Aaron Rowe, a reporter from Chemical & Engineering
News (“C&E News”), at: http://youtu.be/V6gzc8uUGJw. To watch a webinar that discusses FightAIDS@Home and this progress we have made in the last year (followed by a 15 minute Question & Answer period with the public), see http://www.youtube.com/watch?v=khFbQTcoqyI.

**What are “docking calculations”, “virtual screens,” and “drug-resistant mutants”?** Docking calculations are a way to predict how well a small chemical compound can bind to and block the activity of a target protein. We use these calculations to predict the affinity/potency of the compound, the location where it binds on the molecular target, and the mode it uses to potentially disable the target. These predictions help us guide and explain the in vitro experiments (that is, wet lab experiments in test tubes, Petri dishes, etc.) that our collaborators perform. “Virtual screens” are large computational experiments that involve docking collections of hundreds to millions of compounds against particular regions of critical protein targets one at a time. These virtual screens we perform on FightAIDS@Home are like trying to find the right key to open a particular lock on the control panels that regulate the viral machinery. However, both the lock and the keys are flexible—they can change shape (transform their conformations) as they wiggle, jiggle, dance, expand, and contract in the warm watery environments in which they reside. In addition, some locks have multiple different types of keyholes, and only one or two of them might be useful for disabling the viral proteins. When, by chance, the lock’s internal structure happens to change a bit as the virus replicates itself, the potential for evolutionary improvement occurs. If that change in the lock’s guts happens to help the virus escape the effects of a key/drug (while still allowing it to perform its normal biological function), then that new lock becomes a “drug-resistant mutant.” Since HIV copies itself in a very sloppy manner, these accidental changes occur very frequently, which gives HIV the ability to rapidly evolve resistance against the different drugs that are used to fight it.

We are searching for new inhibitors (new keys) that can not only disable the mutant superbugs of HIV that already exist, but that also make it more difficult for the virus to evolve into new drug-resistant mutant forms. Hunting for new keys/drugs is a very tricky and complicated process, because of the reasons discussed previously and the fact that these computational predictions are not perfectly accurate. In addition, the total number of potential “keys” (small organic compounds) that probably exist in the universe (that is, the size of “chemical space”) is estimated to be 10 to the 60th power (that is, 1 with 60 zeros after it). We obviously can not computationally evaluate flexible models of that many different keys, so we have to focus these experiments on the types of keys that are somewhat similar to the types of molecules that have already become approved drugs (or small, representative pieces of these molecules called “fragments”). Instead of searching for a needle in a haystack, we are searching for a needle in many fields full of haystacks, and we can only search through a small percentage of them. To learn more details about these docking calculations, see http://fightaidsathome.scripps.edu/discovery.html or http://gofightagainstmalaria.scripps.edu/index.php/project-details. Thus far, we have received over 168,000 CPU years of free docking calculations on World Community Grid to advance the FightAIDS@Home project.

**What are proteins, what does HIV protease do, and why do we want to block its activity?**

Proteins are polymers that are created by covalently joining (permanently chemically attaching) different peptide units together. Peptides are also called “amino acids”, and there are 20 different flavors of peptides that fall into 3 general categories: charged, polar, and hydrophobic. Since a protein is a polymer made of peptides, it is also called a “polypeptide”. Each type of protein is made of a particular sequence of different peptide units joined together in a specific order to make
a linear chain (somewhat similar to a pearl necklace, if the pearls had very different sizes and chemical properties). After that polypeptide chain is created, the number and order of the different amino acids within it will cause it to fold up into a particular collection, or ensemble, of compact 3-D shapes (conformations). Most human proteins are made as individual, separate polypeptide chains: one polypeptide chain produces one protein. But the components of HIV are made in a very different manner: when the virus’s genetic material directs the infected cell to create HIV proteins, long, multi-protein polypeptide chains are produced.

HIV protease is an “enzyme”. Enzymes (which tend to have names that end in “ase”) are proteins that perform chemical work (such as joining things together, breaking things apart, or modifying specific small organic substances or large biological macromolecules). HIV protease chops the long, viral, multi-protein polypeptide chain at several different, specific places. By cutting that multi-protein chain into different pieces, it then allows those different pieces to fold up into their normal, mature conformations. Those folded viral proteins then work together to create new HIV particles that escape the infected cell (or “bud”), mature, and then infect new cells, which spreads the infection within the patient and allows the infection to spread to new people. When HIV protease activity is sufficiently disrupted, then those multi-protein viral polypeptide chains are no longer separated in an efficient, well-ordered manner. This causes the infected cell to produce immature viral particles that are not able to infect other cells. Of greatest importance, when HIV protease drugs were combined with HIV reverse transcriptase drugs, the death rate associated with HIV infections drastically decreased. Before HIV protease drugs existed, getting HIV was basically a rapid and horrible death sentence. When HIV protease drugs were combined with other classes of anti-HIV drugs to make the HAART cocktails (Highly-Active Anti-Retroviral Therapy), then many HIV patients were able to live long, productive lives with a reasonable quality of life. However, since HIV keeps evolving into slightly different forms that are able to resist the effects of these drugs (that is, new multi-drug-resistant mutant superbugs keep appearing and spreading), we need to discover new types of drugs that can disable these mutants.

**What are allosteric inhibitors?** Most inhibitors disable the function of an enzyme by binding directly to the “active site” (the specific region of an enzyme where the chemical work occurs) and blocking its ability to function. The 9 FDA-approved HIV protease drugs are an example of these conventional types of inhibitors: they bind to and block the active site in the hollow tunnel in the center of protease, which prevents the viral multi-protein polypeptide from being able to bind within that tunnel and get cleaved. Allosteric inhibitors work in a very different way—they bind to a totally different site, regulate the conformational preferences and/or flexibility of the entire target protein, and thereby disable the active site. Allosteric inhibition is like putting a latch on the handles of a pair of scissors in order to prevent the blades from being able to open and close and cut things. Most of the mutations within HIV protease that cause drug resistance generally occur within this active site tunnel; they prevent the drugs from being able to bind tightly, but they still allow the viral multi-protein polypeptide to bind and get chopped into different pieces. The active site of HIV protease is guarded by two highly-mobile flaps, which form the roof of the active site’s tunnel. These flaps must be able to open up to allow the viral multi-protein polypeptide to enter the active site, and they must then be able to close, in order to position the right region of that polypeptide within the active site, so that it binds tightly and creates the right chemical environment that enables the chopping to occur. The flaps must then open up again, to force the cleaved products to escape (and then fold) and to allow a new multi-protein polypeptide (or a new region of the recently-cleaved multi-protein polypeptide) to bind. We are searching for allosteric inhibitors,
because we want to find a way to regulate the opening and closing behavior of the flaps. If the flaps cannot keep opening and closing, then HIV protease cannot function.

Some multi-drug-resistant mutants of HIV protease seem to prefer sampling the conformations that have open flaps more than the normal, “wild type” version prefers them. This makes it more difficult for the drugs to bind tightly to the active site and lock those flaps down into the closed conformation. If we can discover allosteric inhibitors of protease that help close the flaps and/or help keep the flaps closed, then these compounds should help restore the potency of the current protease drugs against these mutant superbugs.

Since mutations randomly occur when the virus replicates itself, it is much more difficult and takes longer for the virus to accumulate mutations in both an allosteric site and the active site of the same viral molecule (in a way that prevents both types of drugs from being able to bind, while still allowing the enzyme to function efficiently). Consequently, the development of new combinations that include active site inhibitors of HIV protease and allosteric inhibitors of protease should not only disable the mutant superbugs of protease that currently exist, it should also impede the evolution of new types of superbugs in the future. There is some evidence to support this ambitious long-term goal: in other systems, such as Bcr-Abl (a target for cancer chemotherapy) and HIV reverse transcriptase, it has been shown that combinations of active site inhibitors and allosteric inhibitors will slow down the development of drug resistance mutations.

**How did we discover these new fragments that bind to HIV protease?**

FightAIDS@Home Experiment 30 involved docking ~ 360,000 different compounds from the “Asinex” library against the two potential allosteric sites that we helped discover previously. See the paper from 2010 that was cited on the bottom of page 2. In effect, these images display the 6 new fragment hits identified in virtual screens against the 1F1 site on top of the flaps. Images E through J show the predicted binding modes of compounds A3, A4, A5, A6, A7, and A8 as yellow spheres on the purple surface representation of protease, while K depicts the superimposition of these 6 hits on the ribbon/cartoon mode of the backbone of HIV protease.
Experiment 30 had two separate halves—the first half involved screening these 360,000 compounds against the potential allosteric site on the top of the flaps, using the 1F1-bound crystal structure of HIV protease as the target. The second half involved docking these 360,000 compounds against the potential allosteric site on the sides of HIV protease, using the 4D9-bound crystal structure of HIV protease as the target. Each half of the experiment was analyzed independently. Computational tools developed in-house by Dr. Stefano Forli (with some help from Dr. Ruth Huey and Associate Professor Michel Sanner) were used to process and then measure the interactions displayed in all of these docking results. These tools were then used to “filter” the results, in order to harvest a small number of top-ranked compounds. Dr. Alex Perryman created a specific set of energetic and interaction-based filters for each of the two sites, and he visually inspected and re-measured the top compounds that passed through each set of filters in order to identify a small set of promising candidate compounds.

For the 360,000 compounds docked against the 1F1 site on top of the flaps, 33 compounds passed through the first set of filters and were visually inspected (using PMV, the Python Molecular Viewer, a program from Associate Professor Michel Sanner’s lab and Professor Art Olson’s lab). Of these 33 top-ranked compounds, we decided to order 10 compounds, so that our collaborators could study them in wet lab experiments. These 10 compounds and the compounds discussed on pages 7-8 were purchased using some of the funds that were donated to FightAIDS@Home from the IBM International Foundation (that is, from part of the award money that IBM’s “Watson” computer won on Jeopardy!; see: http://www-03.ibm.com/press/us/en/pressrelease/33752.wss or http://www.scripps.edu/newsandviews/e_20110228/etc.html). Of these 10 promising compounds, 6 fragments were classified as “hits” according to the results of at least one type of experimental assay. Our collaborators in the Stout lab are currently trying to crystallize these hits with HIV protease, to generate proof regarding where these compounds actually bind and the detailed binding mode that they display at that location. These 6 hits, depicted in images E – K on page 5, have not yet displayed any inhibition of HIV protease activity. They are very small fragments, after all. But 5 of these 6 fragments (A3, A5, A6, A7, and A8) caused a significant shift in the thermal stability of HIV protease in DSF assays (differential scanning fluorimetry) in the Torbett lab, which proves that they are able to bind somewhere on HIV protease (in solution) and affect its overall stability, in a dose-dependent manner (that is, a larger amount of the compound caused a larger change in the thermal stability of protease, which indicates that the compounds bind in a specific manner). Fragment A4 did not cause a significant thermal shift, but it was classified as a hit according to SPR (Surface Plasmon Resonance, which involves analyzing the change in the angle of reflection of a laser that is caused when a compound binds to a target protein that has been pre-attached to a gold or silver surface, see http://en.wikipedia.org/wiki/Surface_plasmon_resonance) experiments performed by the Torbett and Finn labs. Fragment A4 also displayed 53 µM affinity in BSI experiments performed by the Finn lab (backscattering interferometry, a new technique that detects the maximal change in refractive index that occurs when a solute binds to a dilute protein solution; when a compound changes the overall conformational preferences of protease, it causes a change in the refractive index of the solution). BSI data is dose-dependent, as well. Fragments A5 and A8 also displayed low µM affinity in BSI, while fragment A7 displayed > 1 mM affinity in BSI (that is, A7 bound ~ 1,000 times less tightly). The other fragments did not display a good, interpretable signal in BSI. Different types of compounds perform better or worse in different types of experimental assays, which is why our collaborators need to perform many different types of experiments in order to understand how well these compounds actually perform. We are using the
data on these 6 fragment hits to guide the discovery and development of larger, more potent compounds that can hopefully bind the 1F1 site and display inhibition of HIV protease activity. But it still remains to be seen whether binding at the 1F1 site can actually disable the function of the active site.

Of the 360,000 compounds docked against the 4D9 site on the sides of protease, 160 compounds passed through a different set of energetic and interaction-based filters and were visually inspected by Dr. Alex Perryman. We decided to order 34 of these compounds, of which 4 were classified as hits. All 4 of these hits (APex41 to APex44, see images L – O on this page) caused a significant shift in the thermal stability of HIV protease in DSF assays performed by the Torbett lab. APex41 and APex42 displayed 4.3 and 2.7 \( \mu \text{M} \) affinity, respectively, in BSI experiments performed by the Finn lab, while APex43 and APex44 had no detectable signals in BSI. Importantly, all 4 of these hits displayed some inhibition of HIV protease activity in biological assays performed in the Elder lab. At a 100 \( \mu \text{M} \) concentration, APex41 inhibited HIV protease activity by 9\%, while APex42 to 44 inhibited protease activity by 6\%, 2\%, and 3\%, respectively. Although these are very weak inhibitors of HIV protease activity, they are small fragments, and they are the first set of compounds identified in virtual screens against these potential allosteric sites that actually inhibited HIV protease activity in biological assays. But like the other set of hits, we still do not yet have any proof about where these compounds actually bind on HIV protease. Our collaborators in the Stout lab are currently trying to crystallize these hits with protease, to generate that proof regarding where these compounds bind and what interactions they form. We are also going to begin a new type of collaboration with the Kojetin lab at TSRI-Florida that will involve using NMR spectroscopy to provide complementary data regarding where these hits actually bind. We are using the data on these 4 hits, “AutoLigand” fill patterns, and the medicinal chemistry insight provided by the Fokin lab and the Finn lab to guide the design and development of larger compounds that will hopefully bind to this 4D9 site with higher affinity and display more significant inhibition of HIV protease activity.
How are we extending Experiment 30 in order to find larger, more potent inhibitors?

Since the four hits, APex41 – APex44, displayed inhibition of HIV protease activity and were structurally similar to each other, their structural details were used to guide the construction of a “focused library” of ~ 2600 new compounds. Dr. Perryman searched and visually examined the ZINC server (http://zinc.docking.org) to hand-pick these 2600 compounds. Most of these compounds are much larger and more complex than the original APex4_'s; however, some of them have a similar size, but they allow us to explore different Structure-Activity Relationships, in order to determine which types of structural features increase or decrease the potency of these compounds. This focused library of 2600 compounds was then docked against the same 4D9-bound crystal structure of HIV protease, but this time we used our local Linux cluster at TSRI. After applying a similar set of energetic and interaction-based filters, 213 compounds were harvested and had their binding modes visually inspected by Dr. Perryman. During this visual inspection process, the results of “AutoLigand” calculations were used as a guide. AutoLigand is a program from the Olson lab that detects ligand binding sites and describes the general chemical features of compounds that should have maximum affinity for a given volume. See images P and Q and Harris, R., Olson, A.J., and Goodsell, D.S. Automated prediction of ligand-binding sites in proteins. *Proteins*, 70(4): 1506-1517 (2008). We identified and ordered 43 new candidate compounds. These 43 candidates are currently being studied in biological assays performed by the Elder lab. The assays began recently and are still ongoing, but at least 2 of these 43 candidates already displayed some inhibition of HIV protease activity.

Starting from weakly binding fragments and extending them to find much larger, much more potent, drug-like molecules will likely take many different steps and several years. But our journey on this exciting path of discovery has already begun to bear fruit.

While these avenues are being pursued, we are currently screening millions of other compounds against these potential allosteric sites on FightAIDS@Home. Please help us search for allosteric inhibitors of drug targets from HIV by donating your unused computer cycles to World Community Grid.
Dr. Alex L. Perryman wrote Volumes 5 through 11 of the FightAIDS@Home Newsletter. The images displayed in this volume were created by Alex with the Python Molecular Viewer (PMV 1.5.6 release candidate 3, a tool from Associate Professor Michel Sanner’s lab and Professor Art Olson’s lab). Alex thanks Dr. Stefano Forli, Dr. Garrett Morris, and Professor Art Olson for providing proofreading assistance.

We could not perform this much research without World Community Grid’s help or without your donated computer time. Thank you very much for helping us advance the fight against multi-drug-resistant “superbugs” of HIV and for helping us improve the tools and techniques that many other labs use in their own research against other diseases.

We also wish to extend our gratitude to Scott Kurowski and Tim Cusac from Entropia for proposing the initial idea to create the FightAIDS@Home project in 2000, in partnership with Professor Art Olson and Dr. Garret Morris.

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To learn about our new malaria project on World Community Grid, see http://GOFightAgainstMalaria.scripps.edu.

http://fightaidsathome.scripps.edu/