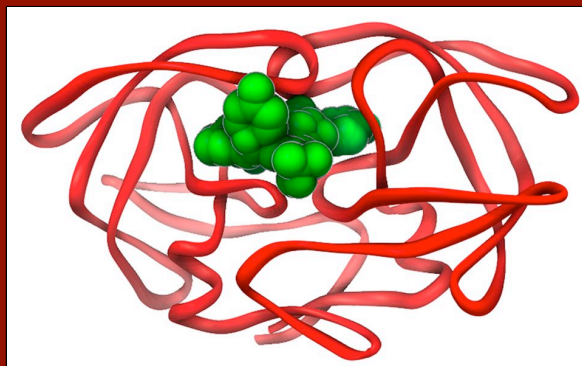
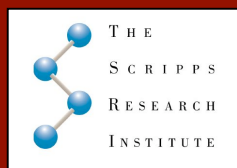


FightAIDS@Home News



Volume 10: October 21, 2011

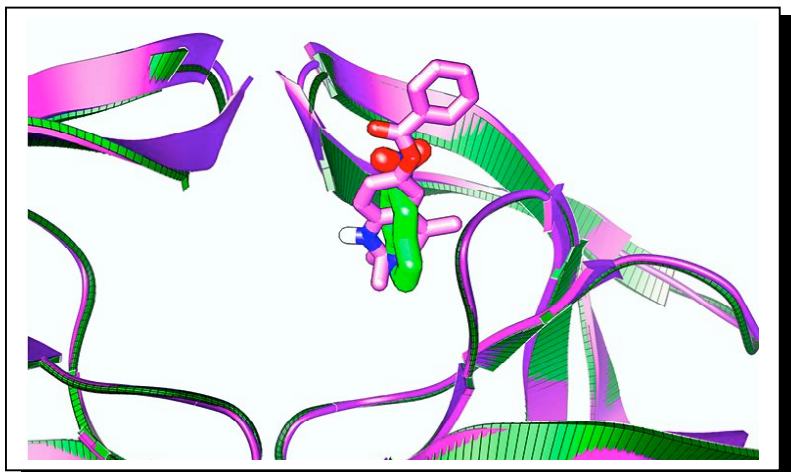
HIV, the virus that causes AIDS, infects over 30 million people throughout the world, and approximately 2 million new people are infected each year. HIV kills more people than any other virus on Earth. Even if/when we can eventually prevent new HIV infections, we will still need to discover new drugs that can 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 recently 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.

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 prefer to bind on the protein target, and (c) what specific interactions are formed between the candidate and the target. That is, 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. Compounds that can bind tightly to the right regions of particular proteins from HIV have the potential to “gum up” 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 two novel, active fragments](#) that are predicted to bind to the active site (where the chemical work occurs) and to the “eye site” of HIV protease (the location near the active site depicted in the image on page 2). Our collaborator Ying-Chuan Lin, in Professor John Elder’s lab, performed the “biological assays” (evaluations of the ability of these compounds to inhibit HIV protease in test

tubes) that showed that these two compounds from Experiment 28 are able to impede HIV protease activity. Read the rest of this newsletter to find out the details of this experiment, how we are following up on it, and what new types of experiments we are currently creating. For a general summary of the FightAIDS@Home project, watch the new YouTube clip of Dr. Alex Perryman's interview by Aaron Rowe, a reporter from Chemical & Engineering News ("C&E News"), at: http://www.worldcommunitygrid.org/about_us/viewNewsArticle.do?articleId=183.

What is the "eye site" and why are we targeting it?



The "eye site" is a little hole that opens up when the flaps that guard the active site are in a semi-open or fully open state. The compounds with green and magenta carbon atoms shown as sticks in the image on the left are bound in the eye site. The eye site is also called the "flap recognition pocket," because when the flaps are closed, the tip of one flap wedges into this region between the other flap and the top of the wall of the active site. If the "eye site" is gummed up with an inhibitor, then the flaps cannot

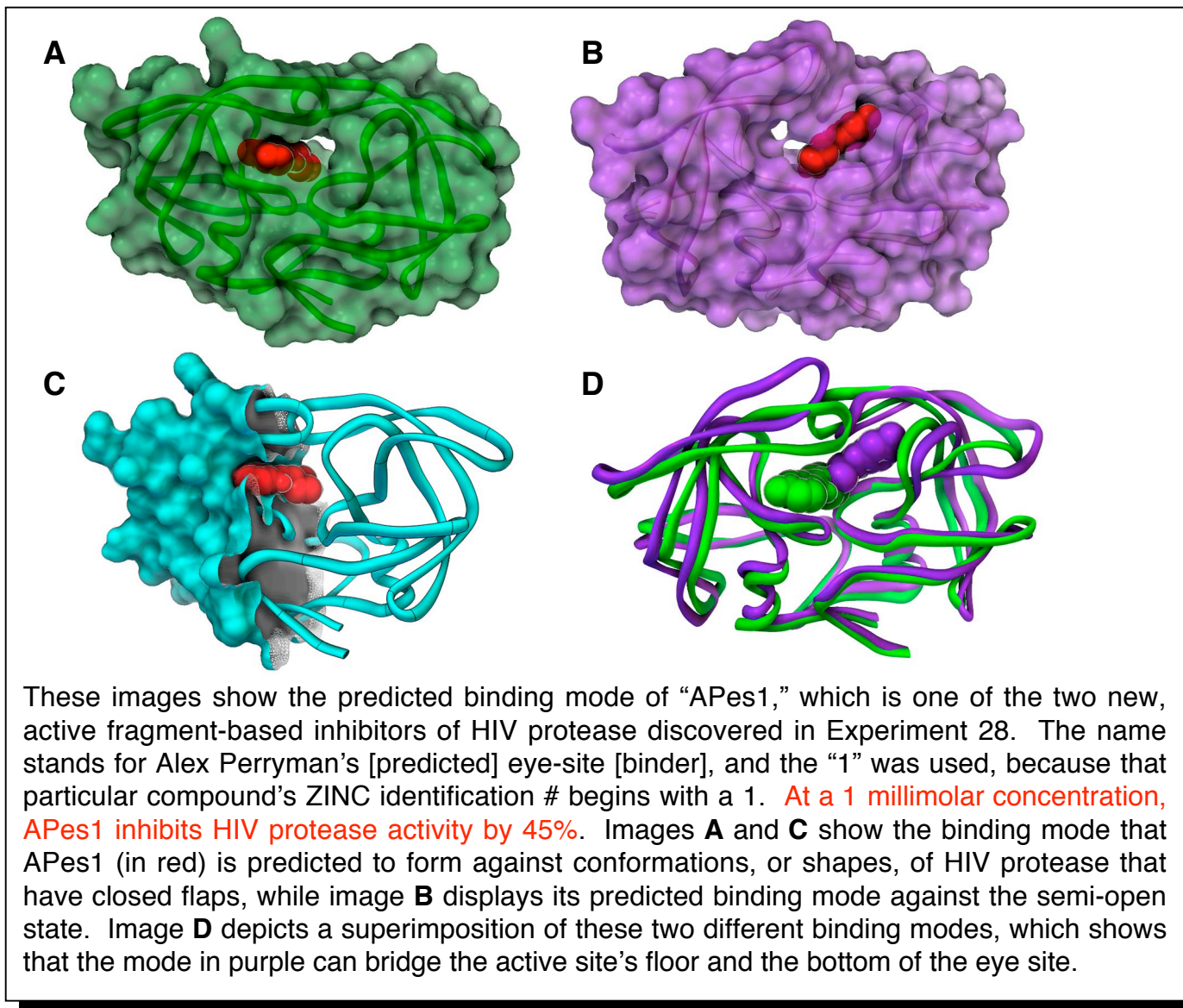
close properly. These flaps must be able to open, close completely, and then re-open repeatedly (like your jaw) in order for HIV protease to perform its chemical work: chopping the newly-synthesized viral multi-protein polypeptide in several specific spots, which separates those viral proteins into their individual units. This allows the viral proteins to fold into their specific, compact 3-D shapes, which allows HIV to construct new viral particles that then disseminate and infect new cells, and, thus, new patients. Hence, compounds that bind to this "eye site" represent a promising new mechanism for inhibiting HIV protease. The idea of targeting this "eye site" was initially championed by Professor Heather A. Carlson's lab in the manuscript "A poke in the eye: Inhibiting HIV-1 protease through its flap-recognition pocket," by Kelly L. Damm and Heather A. Carlson, in *Biopolymers*, **89**: 643-652 (2008). The computationally predicted binding mode of the compound displayed as magenta sticks (Damm compound 1) was from Kelly and Heather's manuscript, while the crystallographic (experimentally determined) binding mode of the compound shown in green (5-nitro-indole) was in the supporting information of our recent paper: "Fragment-based screen against HIV protease," by A.L. Perryman, A.J. Olson, J.E. Elder, C.D. Stout, et al., in *Chemical Biology & Drug Design*, **75**(3): 257-268 (2010).

This eye site is not only a novel region to target on HIV protease, it's also a target that might be able to specifically counteract the mechanisms that some superbugs use to escape the current FDA-approved HIV protease drugs. The Molecular Dynamics simulations that Alex Perryman performed as a graduate student at the University of California, San Diego and the Howard Hughes Medical Institute (see A.L. Perryman, J.H. Lin, and J.A. McCammon, *Protein Science*, 2004) and the series of crystal structures of a wild type (the normal versions of HIV that the drugs still work against), a drug-resistant mutant with 1 amino acid changed, a mutant with 3 changes, and a mutant with 6 changes from our collaborator Professor C. David Stout's lab indicate that

some multi-drug-resistant mutant superbugs of HIV protease seem to have flaps with greater flexibility that prefer to spend more time being semi-open than the wild type. Consequently, compounds that can bind to the eye site, gum it up, and stabilize the flaps in a semi-open state might represent a powerful new approach to fighting these HIV protease superbugs.

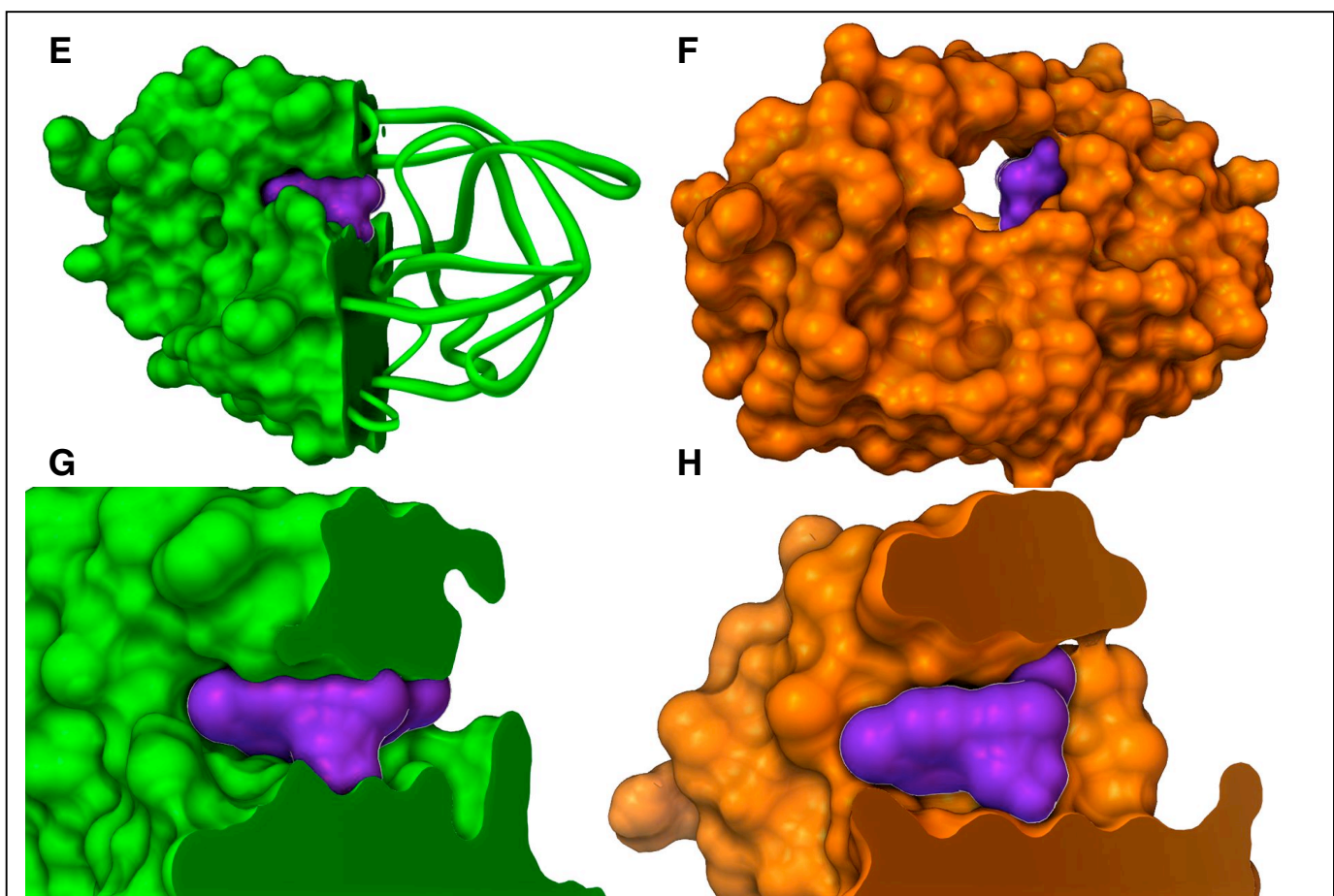
How did we discover these two new fragment-based inhibitors of HIV protease?

FightAIDS@Home Experiment 28 used multiple models of HIV protease that had semi-open flaps and other models that had closed flaps, so that we could target both the eye site and the conventional active site region. We are trying to discover compounds that can bind well to either of these sites, and we are also searching for compounds that might be able to bridge the active site and the eye site (see the red ligand in image **B** below for an example of a bridging compound).



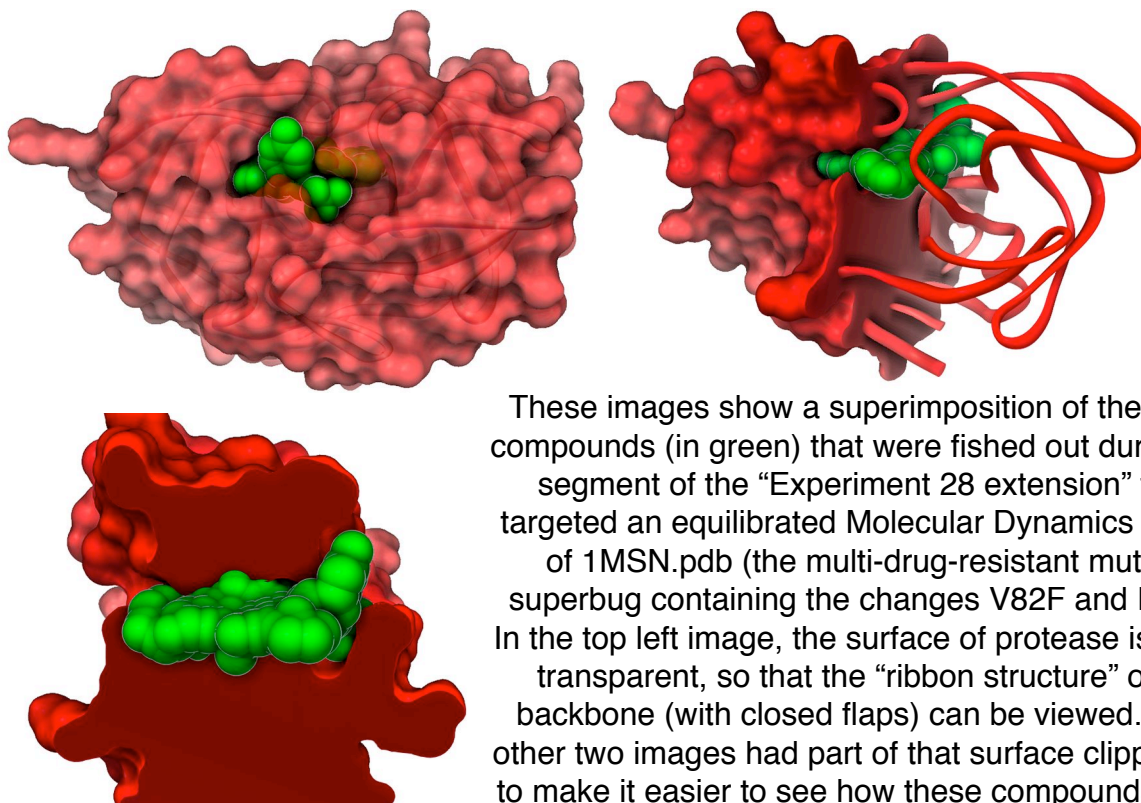
To find these two new fragment-based inhibitors of HIV protease, we took the AutoDock4.2 results from FightAIDS@Home Experiment 28 against an equilibrated Molecular Dynamics output of 1HSI.pdb (HIV-2 protease with semi-open flaps), and we sorted these docking results to harvest compounds for which the lowest energy cluster was also the largest cluster.

For each and every compound that we evaluate on FightAIDS@Home, at least 100 independent docking runs are performed. These different runs for each compound generally produce results that place the compound in different regions of the target, with potentially several different binding modes in each region. We cluster the results of these different runs together, in order to predict which particular binding region and binding mode is most likely to occur. In computational drug design studies, some scientists prefer to focus on the binding mode present in the cluster that displayed the lowest (most favorable) predicted free energy of binding, while other scientists prefer to focus on the cluster that had the largest number of independent runs within it. To avoid this tricky issue, we often just harvest the compounds for which the lowest energy cluster was also the largest cluster, which should hopefully reduce some of the errors that are inherent in all computational experiments.



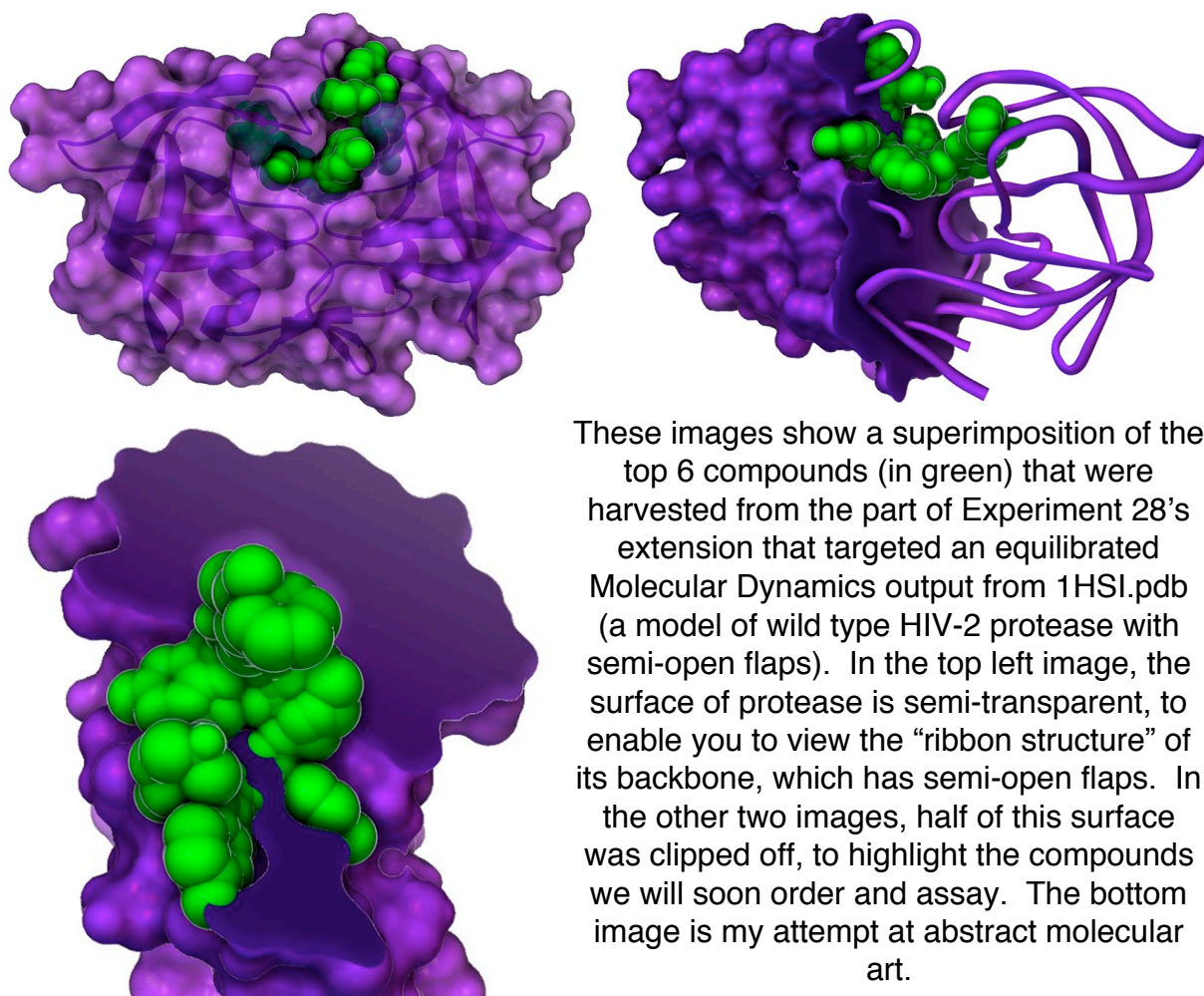
These images display the predicted binding mode for “APes4,” which is the other new, active, fragment-based inhibitor discovered against HIV protease in Experiment 28. **At a 1 millimolar concentration, APes4 inhibits HIV protease activity by 50%.** Images **E** and **G** present the binding mode that this inhibitor is predicted to form against HIV protease conformations that have closed flaps, while **F** and **H** show its predicted binding mode against conformations that have semi-open flaps. When the flaps are semi-open, APes4 (in purple) is predicted to bridge the active site and the eye site.

After sorting the results against 1HSI, 222 compounds were harvested, and Dr. Alex Perryman visually inspected and measured their predicted binding modes. These 222 compounds (from the ChemBridge library of ~ 12,000 “building blocks,” or fragments) were then docked with AutoDock4.2 against a panel of 9 different variants of HIV protease, which included different wild type strains and different drug-resistant mutants, some of which had closed flaps, while others had semi-open flaps. This subsequent round of testing occurred on the Linux cluster at The Scripps Research Institute (TSRI). Similarly, we sorted the results against each target to harvest the compounds for which the lowest energy cluster was also the largest cluster. The top 112 fragments from this round were then docked against the same panel of 9 different variants of HIV protease, but this time we used the new docking program “AutoDock Vina,” which was created by Dr. Oleg Trott and Professor Arthur J. Olson at TSRI. Dr. Alex Perryman then visually inspected the Vina-predicted binding modes of the top-ranked 20 compounds against each of the 9 targets. After all of these different rounds of docking calculations that used two different docking programs, we ultimately decided to purchase the top 10 fragments from ChemBridge. The cost of a few milligrams of each of these 10 fragments (covered by research funds from our collaborator Professor C. David Stout) exceeded a thousand dollars. That’s one of the reasons why drug discovery research is so expensive. These 10 fragments were then assayed in test tubes by our collaborator Ying-Chuan Lin, in Professor John Elder’s lab. Two of these ten fragments were shown to be real inhibitors of HIV protease activity. Although a 20% success rate from these virtual screening results might not sound good, in reality, many of the published results of virtual screening experiments have success rates of only 5 to 10%, and many of the computational scientists in the different drug design groups on LinkedIn say that fragment-based virtual screens are too inaccurate to be of any use. Consequently, we were very pleased to discover two novel fragment-based inhibitors of HIV protease in the results of Experiment 28. And that’s why we continued this line of research in the “extension to Experiment 28” (see images from the extension below).



How are we extending Experiment 28 in order to find larger, more potent inhibitors?

After discovering two new active fragment-based inhibitors in the results of Experiment 28, we created an extension to this experiment. This extension uses the same general protocol that was used in Experiment 28: we docked compounds against that panel of 9 different variations of HIV protease using both AutoDock4.2 and the new program "AutoDock Vina." The main difference is that this extension did not use a particular vendor's library of pre-selected compounds. Dr. Alex Perryman performed a manual, interactive ligand-based search of the ZINC server to find 736 compounds that had some structural similarity to either APes1 or APes4, as well as compounds that contained the "substructure" (small region of the structure that contained key functional groups) that APes1 or APes4 use to bind to the catalytic aspartate residues on the floor of the active site. Since APes1 and APes4 are fragments (a fraction of a drug-like molecule that can be used as an anchor) with millimolar potency against HIV protease, we need to find larger, more complex molecules that are a million times more potent than these two compounds. The 736 compounds in the "focused library" that we generated are larger and more complex than the fragments APes1 and APes4, and we hope that some of these compounds will help us discover new inhibitors that are at least 100 to 1,000 times more potent than these fragments. 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 the journey on this path of discovery has already begun.



Dr. Alex Perryman visually inspected and carefully measured the top-ranked 30 compounds against each of the 9 different variations of HIV protease that were targeted in the extension to Experiment 28. Out of these 270 different computationally predicted binding modes, 32 different compounds displayed promising binding modes and scored well with both AutoDock and Vina. We will soon order these 32 compounds, using some of the money that IBM's "Watson" won on Jeopardy, which was donated to the FightAIDS@Home project (see: <http://www-03.ibm.com/press/us/en/pressrelease/33752.wss> or http://www.scripps.edu/newsandviews/e_20110228/etc.html). As soon as we purchase these 32 compounds, our collaborators in Professor John Elder's lab and Professor Bruce Torbett's lab will perform biological assays to measure their anti-HIV potency.

Some of these 32 promising compounds are displayed in the images on pages 5 and 6. The images of these 32 compounds and of APes1 and APes4 were created in a way that does not reveal their chemical identities. We cannot publicly reveal the identities of these compounds until we have published these results in a peer-reviewed journal. Doing so would prevent us from being able to publish our research. Rules are rules. Our collaborators in Professor C. David Stout's lab are currently trying to crystallize APes1 and APes4 with HIV protease, to prove where and how they actually bind. When they succeed at crystallizing these fragments, we will write and submit a manuscript for publication.

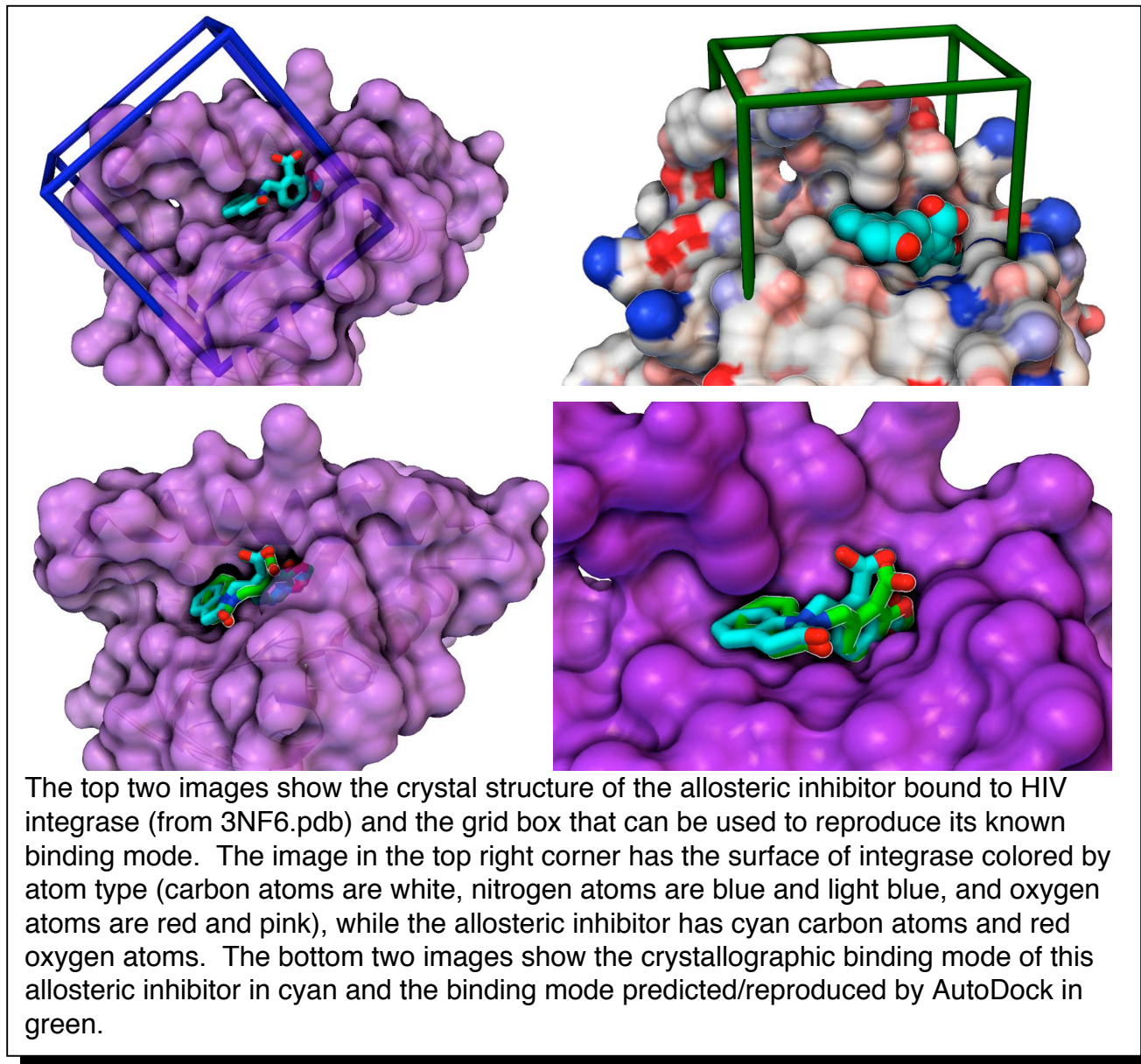
What's coming down the pipeline next?

Other scientists recently discovered an allosteric inhibitor of HIV integrase. To learn more about allostery and its medical significance, see Volume 9 of the FightAIDS@Home Newsletter. The newly-discovered allosteric inhibitor and the atomic-scale 3-D crystal structure of it bound to HIV integrase are from 3NF6.pdb (see <http://www.rcsb.org/pdb>) and the research manuscript "Structural basis for a new mechanism of inhibition of HIV-1 integrase identified by fragment screening and structure-based design," by D.I. Rhodes, T.S. Peat, J.J. Deadman, *et al.*, published in the journal *Antiviral Chemistry and Chemotherapy*, **21**: 155-168 (2011).

In Volume 9 we discussed an early experiment against HIV integrase in which we were hoping to discover an allosteric site by docking compounds against the region underneath the "140s loop". This 140s loop, which is adjacent to the active site, is known to be critical to integrase's ability to function. Although we had the right idea, we now know that we were not targeting the most useful conformations of integrase in that early experiment. Our guess/hope that there might be an allosteric site on HIV integrase was proven to be correct in the research cited above, and we can now target the allosteric-inhibitor-bound conformation of HIV integrase from 3NF6.pdb.

In the experiments that we are currently preparing for FightAIDS@Home, we will be screening millions of compounds against this recently discovered allosteric site that is adjacent to the region underneath the 140s loop. The images on page 8 present the experimentally determined, 3-D crystal structure of this allosteric inhibitor bound to HIV integrase. Before we started creating the input files for these new FightAIDS@Home experiments against HIV integrase, we first performed some "positive control" docking calculations, to make sure that we could use AutoDock to reproduce the known binding mode of this allosteric inhibitor. After adjusting the size and location

of the grid box (the region that the compounds are allowed to explore during the docking calculations) a few times, we were able to successfully reproduce its known, crystallographic binding mode (see the images on page 8). We are using the grid box shown in these images when creating the input files for our new experiments on FightAIDS@Home. **Please help us search for new, larger, more potent allosteric inhibitors of HIV integrase by donating your unused computer cycles to World Community Grid.**

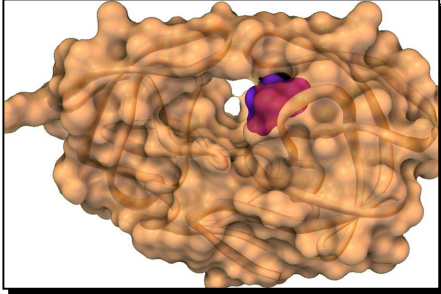


The images displayed in this volume were created by Alex L. Perryman, Ph.D. with the newest version of the Python Molecular Viewer (PMV, a tool from Associate Professor Michel Sanner's lab and Professor Art Olson's lab). Alex thanks Dr. Stefano Forli and Professor Art Olson for providing artistic advice and 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

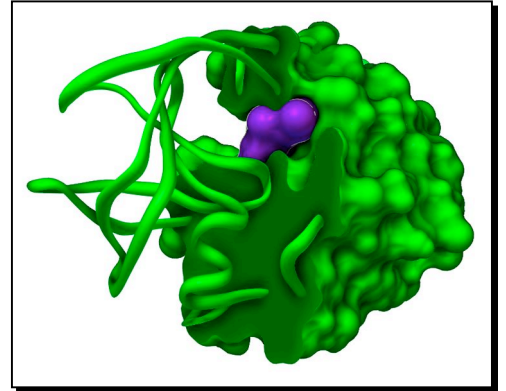
multi-drug-resistant “superbugs” of HIV and for helping us improve the tools and techniques that many other labs use in their own research against other diseases.

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.

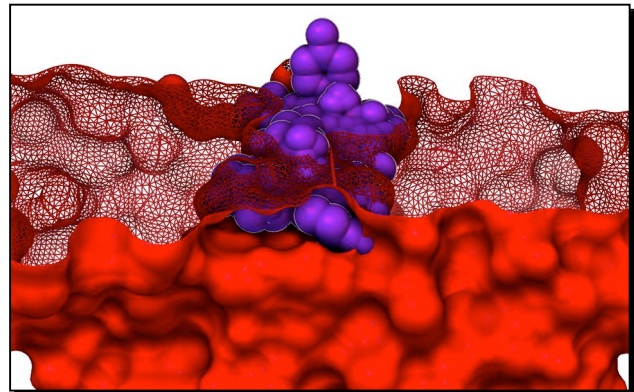
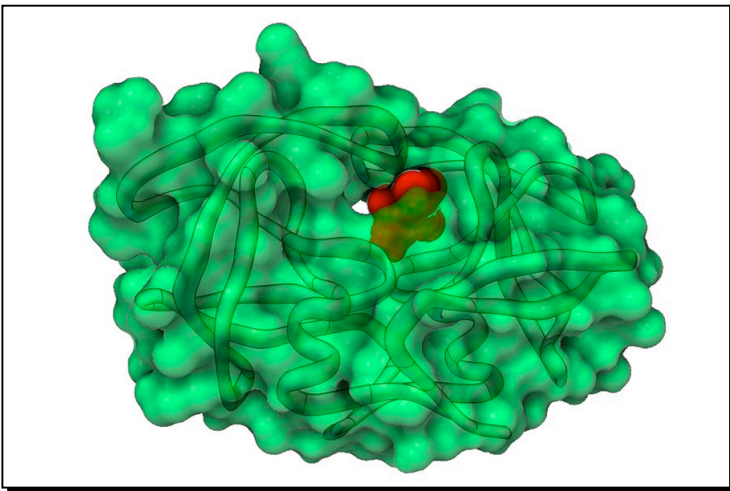


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Dr. Stefano Forli
Dr. Sargis Dallakyan
Dr. Garrett M. Morris
Dr. Ruth Huey
Mike Pique
Professor Arthur J. Olson



Please stay tuned during the next few months to learn about some exciting news regarding our malaria project.



<http://fightaidsathome.scripps.edu/>

