Fragment Screen against HIV Protease: Discovery of Two Allosteric Binding Sites

A team of scientists at The Scripps Research Institute using SSRL resources have applied fragment-based crystallographic screening to HIV protease and discovered two novel binding sites on the surface of the protein. These sites can now be targeted to develop larger, higher affinity drug-like molecules. HIV protease is an essential viral enzyme and important drug target in the fight against AIDS. However, multi-drug-resistant mutations, which severely compromise the potency of protease inhibitors, keep appearing at ever-increasing frequencies [1]. Most mutations that cause drug resistance are found within the active site, in the hollow center of the enzyme, which is guarded by two highly mobile flaps. In contrast, the 'fragments', which are molecules smaller than typical drugs, bind in pockets and clefts on the protease surface and not in the active site. Computer simulations show that one of these clefts must alter its shape when the flaps open and close, as required for catalytic activity during the viral life cycle [2]. Moreover, multi-drug-resistant mutants appear to require a greater range of freedom within this surface binding site [3]. Consequently, larger molecules, designed to bind in these surface sites, should complement active site specific drugs and work to suppress the evolution of resistance by restraining the necessary range of motion in HIV protease. The fragment screening results provide the first key step in this novel approach to drug discovery.

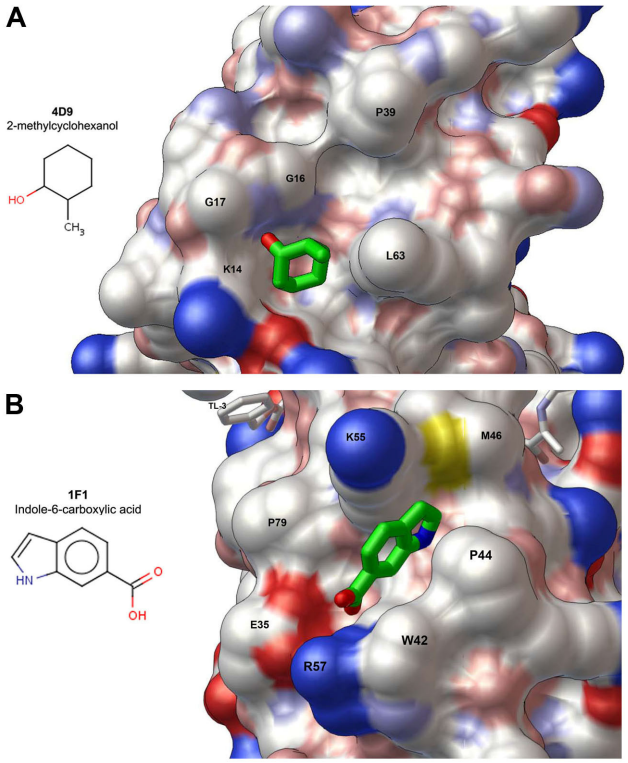

Figure 1. Surface rendering of the HIV protease structure showing solvent-exposed clefts on the protein surface into which the fragments bind. **(A)** The exo site binds 2-methylcyclohexanol, and **(B)** the outside/top of the flap binds indole-6-carboxylic acid. The exo site is a pre-existing feature of the protein fold while the outside/top of the flap rearranges to accommodate fragment binding. These results provide a basis to develop larger, higher affinity inhibitors specific for each site. C, N, O, and S atoms are colored white, blue, red, and yellow, respectively.

HIV protease, with a tight binding inhibitor bound in the active site, was co-crystallized in the presence of small molecule drug fragments. Altogether, 400 fragments were screened, over 800 crystals were evaluated, and 378 data sets were collected to 2.3-1.3 Å resolution using the robotic sample automounter system available at SSRL beam lines. Analysis of the data in collaboration with SSRL staff revealed that fragment binding within each surface site induces a distinct conformation of the protease, leading to appearance of different crystal forms. In the shallow cleft termed the 'exo site' [2, 3] the fragment 2-methylcyclohexanol binds adjacent to the $Gly^{16}Gly^{17}Gln^{18}$ loop where the amide of Gly^{17} is a specific hydrogen bond donor, and hydrophobic contacts occur with the side chains of Lys^{14} and Leu^{63} (Fig. 1A). In a hydrophobic pocket on the outside surface of one protease flap, another fragment, indole-6-carboxylic acid, binds via hydrophobic contacts with Trp^{42}, Pro^{44}, Met^{46},

and Lys[55], a hydrogen bond with Val[56], and a salt-bridge with Arg[57] (Fig. 1B). A similar fragment, 2-acetyl-benzothiophene, also binds at this site. These results could not have been obtained without the high-throughput capability of SSRL beam lines.

This study is the first in which fragments were screened against an inhibitor-bound drug target. The results show that binding sites exist in HIV protease outside the active site and establish a starting point for developing larger, higher affinity molecules able to bind and stabilize the closed, inhibited enzyme. By exploiting an allosteric mechanism such molecules could act in synergy with FDA-approved inhibitors to restore potency against multi-drug-resistant HIV mutants.

Primary Citation

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References

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[2] Perryman, A. L., Lin, J. H. & McCammon, J. A. (2006) Restrained molecular dynamics simulations of HIV-1 protease: The first step in validating a new target for drug design. Biopolymers 82: 272-284.

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