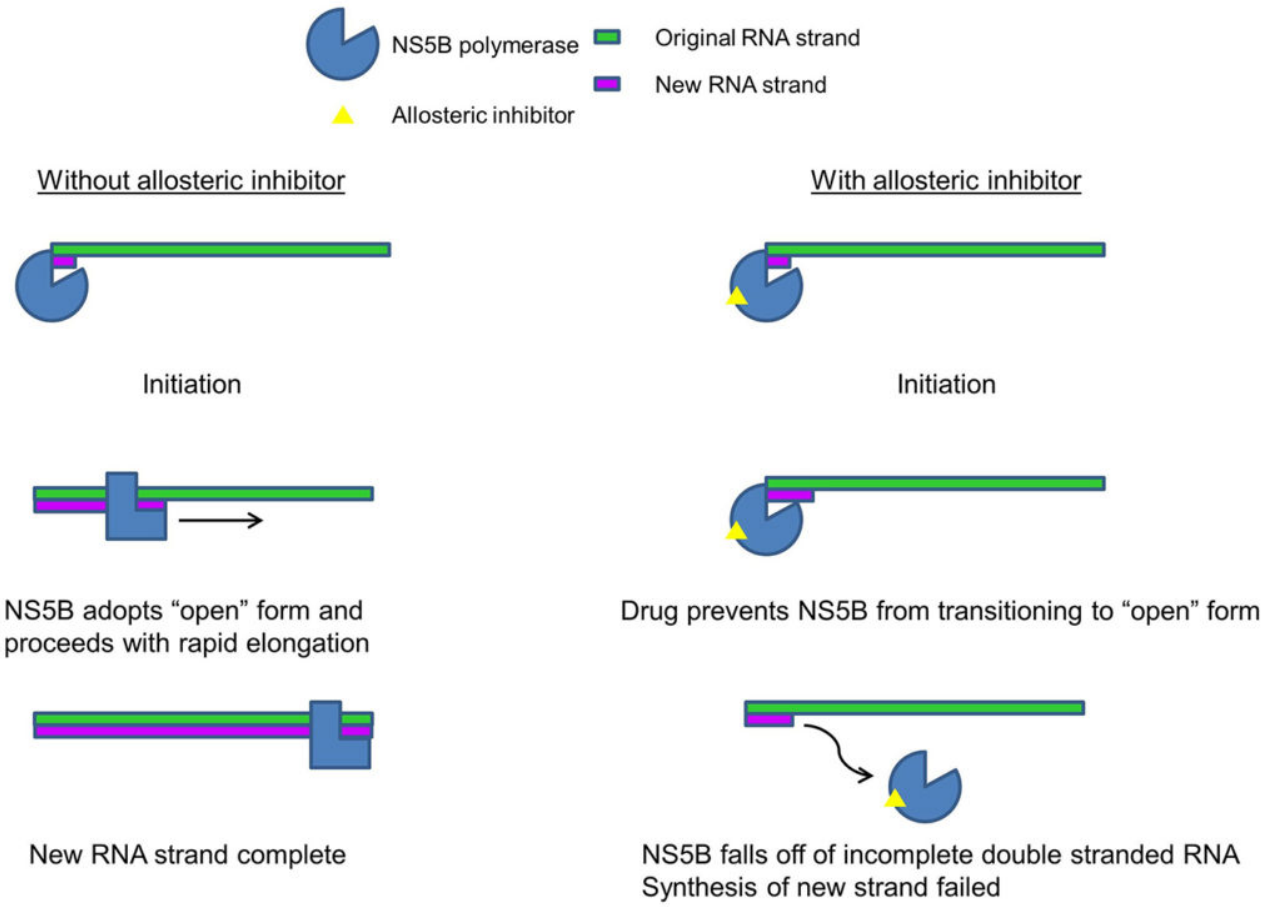
Throwing a wrench into the hepatitis C replication machine

As an essential step in its replication cycle, the human hepatitis C virus (HCV) must make a copy of its RNA genome. The major player in this process is the NS5B polymerase, a molecular machine which uses an existing single stranded copy of the HCV genome as a template for producing a new copy. In developing anti-HCV therapeutics, NS5B has been a major target since blocking NS5B activity blocks an essential step in replication. Several promising HS5B inhibitors currently under development are *allosteric* inhibitors. For enzymes such as NS5B, chemical catalysis takes place at a specific location in the structure called the active site. Many inhibitors act by binding at the active site and preventing the binding of substrate molecules. Allosteric inhibitors, in contrast, bind at sites distant from the active site and exert their inhibitory effects indirectly. Sometimes the allosteric inhibitor causes the target enzyme to undergo a structural change, trapping it in an inactive form. In the case of NS5B, structures of the enzyme with and without bound inhibitors have been solved by X-ray crystallography, and appear to be nearly identical. Images of the static structure, therefore, do not explain how allosteric inhibitors block NS5B activity.



Mechanism of NNI-2 Inhibitors of HCV Polymerase

Like all machines, molecular machines such as NS5B must move as they perform their functions. We proposed that allosteric inhibitors interfere with NS5B function by suppressing molecular motions that are essential for the replication cycle. To test this, we employed an isotope exchange ("hydrogen deuterium exchange") technique coupled with mass spectrometry. This technique allows us to "image" which parts of a protein molecule are mobile and flexible and which parts are immobile and rigid. We found that, when a specific class of allosteric inhibitors, called NNI-2 inhibitors, bind to NS5B, formerly mobile regions throughout much of the molecule become rigid. Repeating these measurements using a series of NNI-2 inhibitors of different potencies, we found that the more potent inhibitors caused more extensive rigidification of the molecule.

Why would loss of mobility prevent NS5B from replicating viral RNA? The answer lies in NS5B's complex replication cycle. In the first stage of replication, NS5B binds to single stranded RNA and begins the synthesis of a new strand by adding complementary nucleotides. To accomplish this, NS5B must adopt a compact "closed" structure in order to correctly bind the single stranded template RNA. After this initiation stage, NS5B continues to synthesize the new complementary RNA strand, but after adding the first few nucleotides, it must change its shape and adopt an "open" structure in order to accommodate the growing double stranded RNA chain. Once this structural change takes place, the reaction proceeds to the "elongation" stage, where NS5B rapidly completes the synthesis of the new RNA stand. If NS5B fails to accomplish this structural change, then replication is aborted, and the result is a short incomplete stretch of 5-6 nucleotides instead of a complete new RNA strand. Using careful measurements of the enzymological activity of NS5B, we have shown that NNI-2 inhibitors prevent NS5B from transitioning to the elongation stage of the replication reaction, and our measurements on the mobility of NS5B indicate that these allosteric inhibitors "freeze out" the molecular mobility that allows NS5B to transition to an open structure. Instead of blocking the active site, as more conventional inhibitors do, NNI-2 inhibitors of NS5B lock it into a closed structure, so it can initiate the reaction by binding to the single stranded RNA template but cannot complete the synthesis of the new strand.

Now that we understand how NNI-2 drugs block HCV replication, scientists can use this knowledge to design more effective allosteric inhibitors.

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Publication

Hydrogen/Deuterium Exchange Kinetics Demonstrate Long Range Allosteric Effects of Thumb Site 2 Inhibitors of Hepatitis C Viral RNA-dependent RNA Polymerase.

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