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Computational predictions suggest that structural similarity in viral polymerases may lead to comparable allosteric binding sites

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ABSTRACT

The identification of ligand-binding sites is often the first step in drug targeting and design. To date there are numerous computational tools available to predict ligand binding sites. These tools can guide or mitigate the need for experimental methods to identify binding sites, which often require significant resources and time. Here, we evaluate four ligand-binding site predictor (LBSP) tools for their ability to predict allosteric sites within the Hepatitis C Virus (HCV) polymerase. Our results show that the LISE LBSP is able to identify all three target allosteric sites within the HCV polymerase as well as a known allosteric site in the Coxsackievirus polymerase. LISE was then employed to identify novel binding sites within the polymerases of the Dengue, West Nile, and Foot-and-mouth Disease viruses. Our results suggest that all three viral polymerases have putative sites that share structural or chemical similarities with allosteric pockets of the HCV polymerase. Thus, these binding locations may represent an evolutionarily conserved structural feature of several viral polymerases that could be exploited for the development of small molecule therapeutics.

1. Introduction

Over the past two decades, there have been many advances in biochemical and biophysical techniques that allow us to identify the proteins responsible for human diseases. An important factor in treating many of these diseases is identifying sites on the corresponding proteins where small molecules may bind in order to modulate protein function. Unfortunately, sometimes it is difficult to determine the three-dimensional structure and location of binding sites with experimental techniques such as X-ray crystallography (Hassell et al., 2007). In such scenarios computational methods may be of significant utility. Over the past decade we have seen an increase in the number of computational methods available to predict protein binding sites. Many of these methods can be used in combination with experimental studies as a validation tool or to guide experimental design. With these considerations in mind, we have assessed the ability of four Ligand Binding Site Predictors (LBSPs) to predict known binding sites within the Hepatitis C virus (HCV) polymerase. The most successful LBSP was then used to identify novel binding sites on the surfaces of three structurally related but less well-studied viral polymerases.

Viral polymerases are crucial players in the life cycle of viruses and are often validated targets for therapeutics (De Clercq, 2005; Sesmero and Thorpe, 2015). Consequently, our studies have broad implications in suggesting new locations on viral polymerases that can be targeted by small molecules and thus new therapeutic strategies for viral infections. The HCV polymerase is of particular interest, as it displays multiple binding sites for different classes of small molecule inhibitors. Thus, it serves as a useful and interesting model system with which to test the efficacy of binding site prediction algorithms. HCV infection continues to be a global health concern, affecting approximately 200 million people worldwide (Seff and Hoofnagle, 2002; Beaulieu, 2009). The therapeutic landscape for HCV infection has improved significantly in recent years with the introduction of new and more effective therapies (Lawitz et al., 2013; Isaac et al., 2015; Keating, 2015). However, these therapies continue to have limitations including high cost. The HCV polymerase is one of the key enzyme targets of small molecule therapeutics approved to treat HCV infection and is an ongoing focus of drug discovery efforts. In addition to the active site, the enzyme possesses four allosteric sites that can be targeted for enzyme inhibition (Beaulieu 2009; Li et al., 2009). The HCV polymerase possesses the canonical "right hand" structure common to viral polymerases, consisting of the fingers, thumb and palm subdomains. However, it resembles a "closed hand" rather than the "open hand" configuration frequently observed in other

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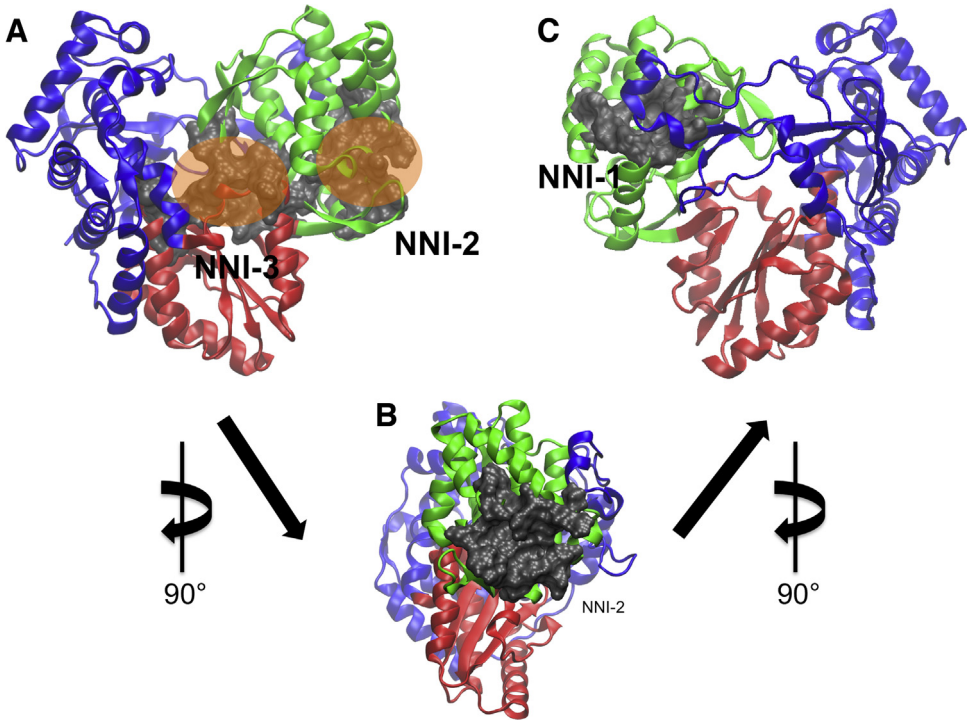

Fig. 1. Allosteric sites in the HCV polymerase used to evaluate the LBSPs. Protein residues are shown in ribbon representation while residues lining the binding pockets are shown in grey surface representation. Panel A shows a frontal view of the NNI-2 and NNI-3 sites, which are differentiated by the positions of orange translucent spheres. Panel B is a magnified lateral view of the NNI-2 site rotated 90° about the y-axis. Panel C is a rear view of the protein depicting the NNI-1 pocket after an additional 90° rotation about the y-axis. The thumb, palm and fingers domains are shown in green, red and blue respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

viral polymerases. The active site and two allosteric sites are found within the palm domain, while the remaining two allosteric sites are located in the thumb domain (Fig. 1). A range of small molecules with diverse chemical properties have been discovered and optimized to target these sites (Condon, 2005; Beaulieu, 2007; Burton and Everson, 2009). Active site inhibitors are either nucleoside inhibitors (NIs) or pyrophosphate analogs, while those that target the allosteric pockets are termed nonnucleoside inhibitors (NNIs) (Condon, 2005; Beaulieu, 2007; Burton and Everson, 2009). Consequently, the allosteric binding sites are referred to as NNI pockets (Fig. 1).

The strong structural similarities shared by the polymerases of HCV and other viruses suggest that other viral polymerases may exhibit allosteric sites analogous to those observed in the HCV enzyme. If so, it may be possible to identify novel small molecules that could inhibit these enzymes in a similar manner to that achieved for HCV. This is particularly important for viruses that do not currently have therapeutic options available. In this study, we examined polymerases of Dengue (DENV), West Nile (WNV) and Foot-and-mouth disease (FMDV) viruses in order to predict the location of potential allosteric binding sites. These diseases have no or limited treatment options available (Malet et al., 2008; Noble et al., 2010) and have become increasingly prevalent, cause significant mortality, morbidity or economic cost. The first major obstacle encountered in performing a study such as this is that there is not much biochemical or structural information identifying allosteric sites within these polymerases. This makes the problem well suited for the application of computational tools to predict novel binding sites within these proteins. In doing so, we have placed emphasis on allosteric sites for two main reasons: i) active sites are well conserved across many viral families, thus predicting active site binding is not anticipated to be difficult and ii) in drug discovery for polymerases, allosteric sites tend to be unique to the virus. Thus, inhibitors that bind at these sites may reduce nonspecific side effects that can lead to host cellular toxicities. We believe that the HCV polymerase is an excellent model to evaluate the effectiveness of ligand binding site predictor (LBSP) tools in predicting allosteric sites due to the wealth of structural and biochemical data available describing the interactions between allosteric inhibitors and this enzyme. Additionally, we suggest that one can use existing information available for the HCV polymerase to make meaningful inferences about the locations of allosteric sites in the DENV, WNV and FMDV polymerases due to the strong structural and functional similarities among these enzymes.

Four different LBSPs were employed to determine which tool performed best at predicting allosteric binding sites within the HCV polymerase. Our target sites are: NNI-1 and NNI-2 located in the thumb and NNI-3 found in the palm (Fig. 1). Because the two allosteric sites found within the palm domain (NNI-3, NNI-4) largely overlap, we treated these as a single site, using the residues for NNI-3 as a proxy for both palm allosteric sites given that NNI-3 spans the palm domain and thumb-fingers junction. Additionally, our previous studies have shown that an NNI-3 inhibitor is able to interact with diverse protein residues throughout the palm and thumb domains, including residues that are typically considered part of the NNI-4 pocket. This observation suggests that the NNI-3 and NNI-4 sites are not distinct binding locations but instead are different regions within a single, large binding pocket in the palm domain (Brown and Thorpe, 2015). Targeting these three allosteric sites with diverse residue composition, size, shape and location allows for a robust evaluation of the LBSPs in identifying allosteric binding sites in viral polymerases. The tools evaluated were: FTSite (Ngan et al., 2012), QsiteFinder (Laurie and Jackson, 2005), LISE (Xie and Hwang, 2012) and Ligsite^{csc} (Huang and Schroeder, 2006). For this study, we focused on allosteric sites in the HCV polymerase because these pockets have been validated as drug targets