	Experiment title: Structural studies of HCV polymerase (genotype 1b) in complex with allosteric inhibitors	**Experiment number**: MX-267
Beamline: ID14 1 ID14 4	**Date of experiment**: from: 30 October 2004 to: 1 November 2004 from: 25 November 2004 to: 26 November 2004	**Date of report**: 20-July-2005
Shifts: 6 3	**Local contact(s)**: Joanna Timmins Raimond Ravelli	*Received at ESRF:*

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Stefania Di Marco* and Andrea Carfi'*

Report:

1. J Biol Chem. 2005 Jun 13; [Epub ahead of print]

Interdomain communication in hepatitis C virus polymerase abolished by small-molecule inhibitors bound to a novel allosteric site.

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The hepatitis C virus (HCV) polymerase is required for replication of the viral genome and is a key target for therapeutic intervention against HCV. We have determined the crystal structures of the HCV polymerase complexed with two indole-based allosteric inhibitors at 2.3 A and 2.4 A resolution. The structures show that these inhibitors bind to a site on the surface of the thumb domain. A cyclohexyl and phenyl ring substituents, bridged by an indole moiety, fill two closely spaced pockets whereas a carboxylate substituent forms a salt

bridge with an exposed arginine side chain. Interestingly, in the apoenzyme, the inhibitor binding site is occupied by a small alpha-helix at the tip of the N-terminal loop that connects fingers and thumb domains. Thus, these molecules inhibit the enzyme by preventing formation of intramolecular contacts between these two domains and consequently precluding their coordinated movements during RNA synthesis. Our structures identify a novel mechanism by which a new class of allosteric inhibitors inhibits the HCV polymerase and open the way to the development of novel antiviral agents against this clinically relevant human pathogen.

2. J Med Chem. 2005 Jul 14;48(14):4547-57

Potent Inhibitors of Subgenomic Hepatitis C Virus RNA Replication through Optimization of Indole-N-Acetamide Allosteric Inhibitors of the Viral NS5B Polymerase.

Harper S, **Avolio S**, **Pacini B**, **Di Filippo M**, **Altamura S**, **Tomei L**, **Paonessa G**, **Di Marco S**, **Carfi A**, **Giuliano C**, **Padron J**, **Bonelli F**, **Migliaccio G**, **De Francesco R**, **Laufer R**, **Rowley M**, **Narjes F**.

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Infections caused by hepatitis C virus (HCV) are a significant world health problem for which novel therapies are in urgent demand. Compounds that block replication of subgenomic HCV RNA in liver cells are of interest because of their demonstrated antiviral effect in the clinic. In followup to our recent report that indole-N-acetamides (e.g., 1) are potent allosteric inhibitors of the HCV NS5B polymerase enzyme, we describe here their optimization as cell-based inhibitors. The crystal structure of 1 bound to NS5B was a guide in the design of a two-dimensional compound array that highlighted that formally zwitterionic inhibitors have strong intracellular potency and that pregnane X receptor (PXR) activation (an undesired off-target activity) is linked to a structural feature of the inhibitor. Optimized analogues devoid of PXR activation (e.g., 55, EC(50) = 127 nM) retain strong cell-based efficacy under high serum conditions and show acceptable pharmacokinetics parameters in rat and dog.

3. Another paper which includes the structure with other allosteric inhibitors that still bind in the thumb domain of the polymerase is in preparation

PDB codes:
2brk and 2brl