

MECHANISM OF IVERMECTIN-MEDIATED FLAVIVIRAL HELICASE INHIBITION

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INTRODUCTION

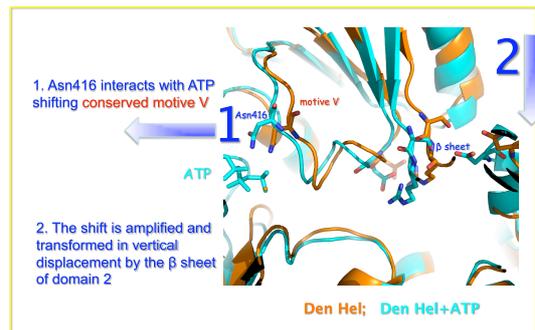
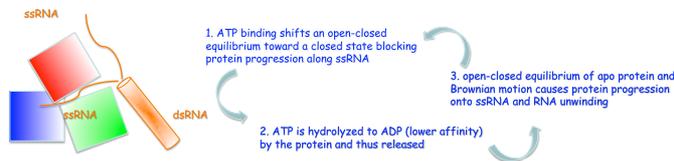


Flavivirus infections have a significant impact on the health of people worldwide. Infection with yellow fever virus (YFV), the prototypic mosquito-borne flavivirus, causes severe febrile disease with high mortality. Moreover, in recent years, the *Flavivirus* genus gained further attention due to re-emergence and increasing incidence of West Nile, Dengue and Japanese encephalitis viruses. There is thus an urgent need for antiviral drugs to treat life-threatening infections with flaviviruses.

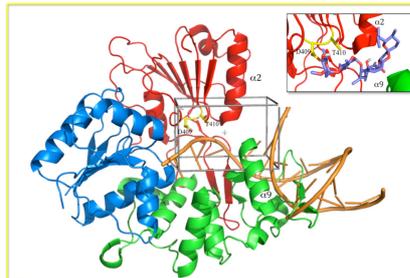


MOLECULAR DYNAMIC SYMULATION

In order to investigate Hel dynamical features we performed two molecular dynamics simulations of Dengue virus Hel (pdb 2JLQ; Luo et al., 2008) in the presence and absence of ATP. The starting model produced to simulate the protein dynamics in the presence of ATP was identical to the apo model but for the addition of the ligands (ATP and Mg²⁺) and different conformation of 24 amino acids (184-208) modelled from the AMPPNP bound structure (pdb 2JLR).

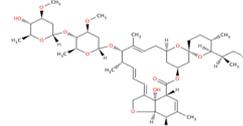


The MD simulation showed that ATP induces the closure of the ssRNA access site. The mechanical feature of the signal transmission between ATP binding site and ssRNA access site, located about 30 Å apart, are described by a novel role of Hel motif V (Mastrangelo et al., 2012).



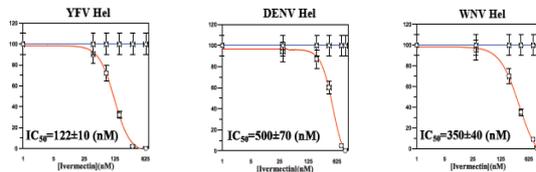
TARGET-BASED IDENTIFICATION OF INHIBITORS

Starting from the high resolution crystal structure of the NS3 helicase from West Nile virus we identified a novel protein site that potentially could be targeted for enzyme inhibition. *In silico* docking of a library of small molecules allowed us to identify compounds with high predicted affinity for the new site; among these the broadly used anti-helmintic drug Ivermectin.



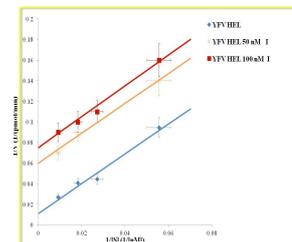
MUTATIONAL ANALYSIS OF PUTATIVE IVERMECTIN BINDING SITES

We report Ivermectin inhibition curves of YFV-double mutant (Thr413Ile and Asp414Glu), DENV double mutant (Thr408Ile and Asp409Glu) and WNV-double mutant (Thr409Ile and Asp410Glu) (blue lines) and the corresponding wild type enzymes (red lines) showing that mutations of putative Ivermectin binding residues of flaviviral helicases abolish Ivermectin inhibition (blue lines).



INHIBITION MECHANISM

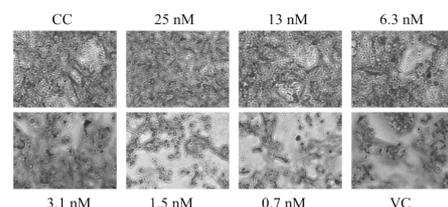
In order to dissect the mechanism of Ivermectin-mediated helicase inhibition on the molecular level we performed enzymatic assays using flavivirus helicases and increasing amounts of Ivermectin and dsRNA substrate and we demonstrated that Ivermectin acts as an uncompetitive inhibitor against flaviviral helicases (Mastrangelo et al., *in press*). No activity was observed against i.e. HCV helicase.



	YFV Hel	DENV Hel	WNV Hel
K _i (μM)	0.019 ± 0.002	0.354 ± 0.023	0.175 ± 0.025

ANTIVIRAL ACTIVITY OF IVERMECTIN

Ivermectin was shown to possess an anti-YFV activity by both a CPE reduction assay (EC₅₀ = 7.4 nM;) and a virus yield reduction assay (quantification by RT-PCR; EC₅₀ = 0.5 nM). Ivermectin is specifically effective against the genus *Flavivirus* (i.e. Dengue fever; EC₅₀ = 700 nM, Japanese encephalitis EC₅₀ = 300 nM and tick-borne encephalitis viruses; EC₅₀ = 200 nM). No antiviral activity was observed against other viruses. Time of drug addition experiments showed that Ivermectin inhibits YFV and DENV production at a time point matching the onset of intracellular viral RNA synthesis, confirming viral RNA replication as inhibition target.



IVERMECTIN IS SPECIFIC against FLAVIVIRUSES

Vero-E6	JEV	TBEV	Vero76	HSV-1	VV	VSV	CVB 2	Sb-1	RSV
%CC	%EC ₅₀	%EC ₅₀	%CC	%EC ₅₀					
10	0.3	0.2	6	>6	>6	>6	>6	>6	>6

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