Virtual screening for *Citrus Tristeza Virus*

[1]A. Lombardo, [1]G. Licciardello, [2]G. Vistoli, [1]A. Bertuccio, [2]A. Pedretti

[1]Parco Scientifico e Tecnologico della Sicilia, Z.I. Blocco Palma I, Stradale V. Lancia 57 95121, Catania.
[2]Dipartimento di Scienze Farmaceutiche "Pietro Pratesi", Università degli Studi di Milano, Via L. Mangiagalli 25, 20133 Milano

Citrus tristeza virus (CTV) causes a most destructive citrus disease in many parts of the world and several approaches have been used to control damages like eradication programs, use of tolerant or resistant rootstock, use of transgenic *Citrus* and cross protection. The aim of this study was to identify, by a computational approach, molecules that interfere with CTV replication. Drug discovery is an extended process that can take as many as 15 years from the first compound synthesis in the laboratory until the therapeutic agent, or drug, is brought to market. GriDock is a molecular modelling software developed to identify potentially bioactive compounds (hit-compounds) in order to speed-up the drug discovery process. It's based on the virtual-screening approach in which the activity of a large set of molecules is predicted by multiple molecular docking calculations. With a view to verify its potentialities, the RdRp (RNA dependent RNA polymerase) enzyme of CTV was chosen as a potential target. RdRp 3D structure was obtained through homology approaches. The screening of more than one million of molecules allowed the identification of some potential RdRp inhibitors. Sulfonic acids and penicillin derivatives were selected and an *in vitro* assay to test their activities was developed. Results must be considered preliminary but of great interest for a novel and innovative approach to study CTV replication rate, biology and plant interactions.

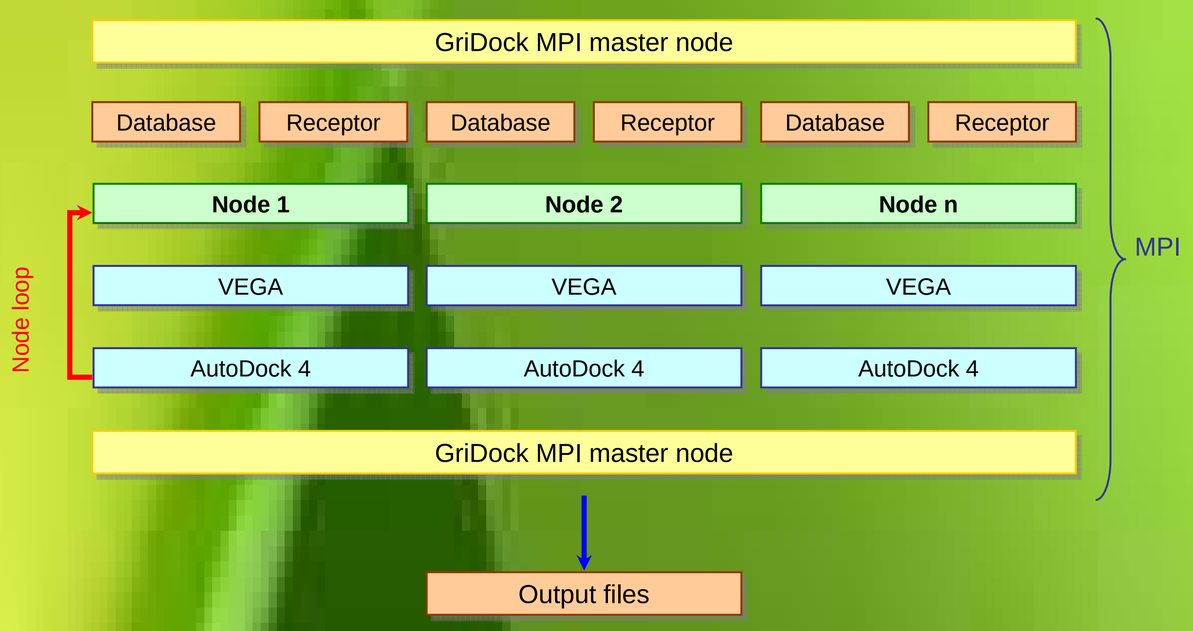

Fig. 1 GriDock combines the VEGA flexibility and the AutoDock 4 power to take full advantage of the Grid technology

Experimental details

Citrange troyer discs leaf samples (2mm^2) infected with SG29 isolate, were maintained in liquid medium, in growth chamber for 14 days with 20000 lux for 16 hours at 28℃, 8 hou rs dark at 23℃, humidity 70%. Two selected drugs were test ed in three different concentrations (0,1%, 0,2%, 0,3%). No treated leafs were used as internal control. RNAs were isolate and the cDNAs were synthetized.

Real-Time PCR

Replication rate of CTV was tested by the comparative Ct method (DDCT). As reference gene, eukaryotic elongation factor was chosen. For CTV relative quantification partial UTR region was amplified using primers and probe described by Bertolini *et al.* (2008) [3].

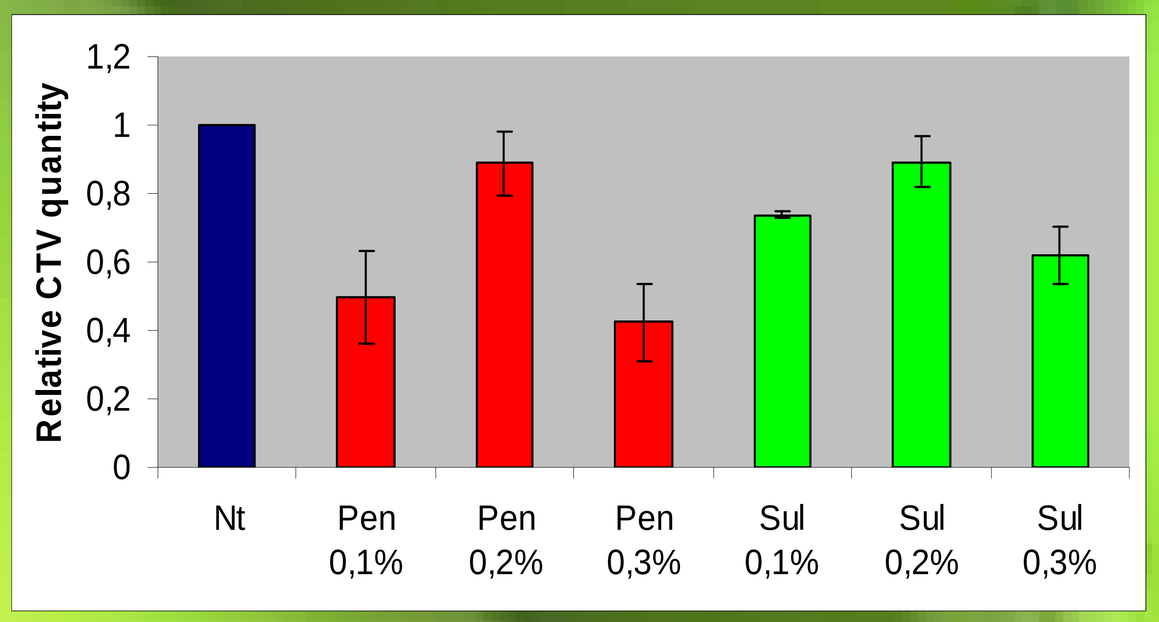

Fig. 3 Inhibition of viral replication rate by real-time PCR. Nt: not treated; Pen: penicillin derivative (0,1-0,2-0,3% w/v); Sul: sulfonic acid derivative (0,1-0,2-0,3% w/v)

Computational details

- We developed a new parallel *structure-based virtual screening* software (*GriDock*) able to run on both multi-CPU and GRID systems
- It's a front-end to the well known *AutoDock* software, developed by D.S. Goodsel and A.J. Olson
- It uses VEGA command-line software to perform file format conversion, database extraction and molecular property calculations
- It can take full advantages of multi-CPUs/cores systems and GRID-based architectures through its parallel design (Fig.1). We screened about **1 million molecules** included in the ligand info database (http://ligand.info) principally derived from drugs.

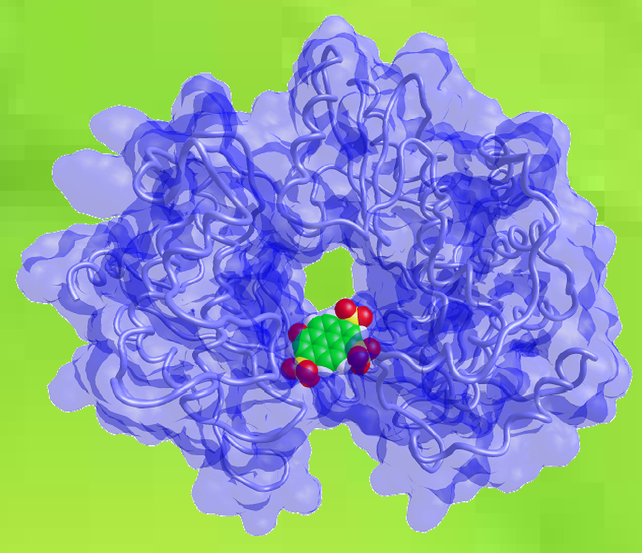

Fig.2 Binding of a sulfonic acid on RdRp

Results

- The complete RdRp model required by GriDock was obtained by the homology modeling approach
- Selected molecules target contain sulfur atoms and multiple sulfonic acid moieties
- Some of them are included in the Anti-HIV class
- **A sensible inhibition of viral replication was obtained**

  Results obtained by *in vitro* tests, although in a preliminary phase, confirmed the *in silico* approach results. In the next future molecules have to be tested in plants for *in vivo* exsperimentation. More compounds and CTV proteins could be explored.

References: [1] N. Duran-Vila, M. Cambra, J. A. Pina, J. F. Ballester and L. Navarro (1988). In Proc. 10th Conf: IOCV; [2] A. Pedretti, A. Lombardo, G. Vistoli, E. Mastriani, F. Pappalardo and S. Motta (2009); Final workshop of GRID projects PON ricrca 2000-2006. Avviso 1575.; [3] E. Bertolini, A. Moreno, N. Capote, A. Olmos, A. de Luis, E. Vidal, J. Pérez-Panadés, M. Cambra (2008) Eur J Plant Pathol.

2nd International Citrus Biotechnology Symposium, Catania, Italy, november 30 - december 2, 2009