A multidisciplinary approach for the identification of Tsg101-UEV ligands with potential as novel broad-spectrum antivirals

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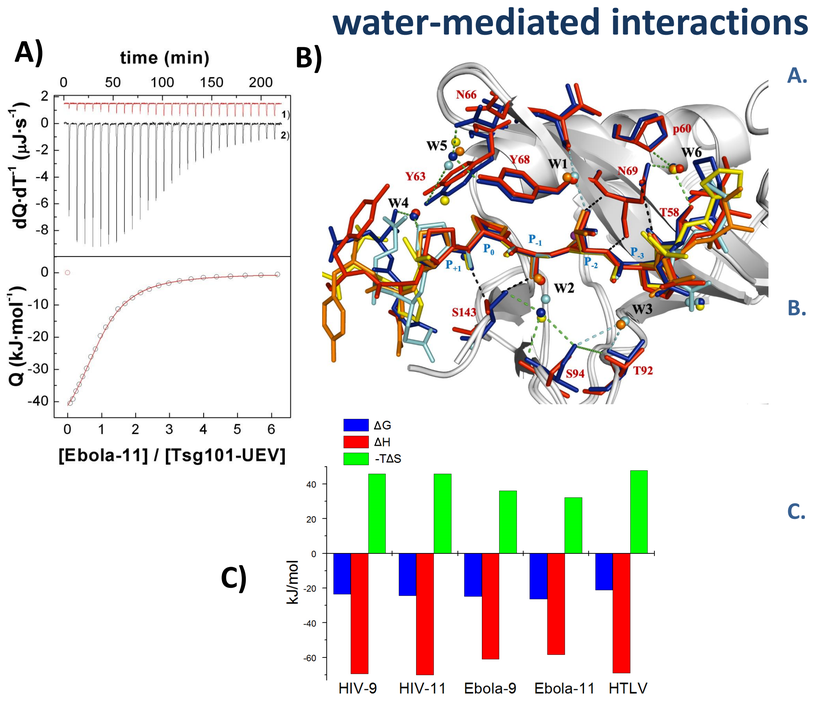
ABSTRACT.- The recognition of PTAP viral Late domains by the UEV domain of the human Tumor Susceptibility Gene 101 (TSG101-UEV) is essential for the budding of many viruses such as Ebola, HIV or HTLV. Blocking TSG101-UEV/Late domain interactions has been shown to stop virus release. We have set-up a multidisciplinary approach combining biophysical studies with phage display and high-throughput screening methodologies in search for ligands of TSG101-UEV with potential as novel broad-spectrum antivirals. We present here a detailed structural, thermodynamic and molecular dynamics study of the binding of TSG101-UEV to a set of peptide ligands derived from the Late domains of HIV, Ebola and HTLV and a set of high-affinity peptides obtained by phage display, which has provided a good understanding of the molecular determinants of these interactions. Also, to explore the druggability of the TSG101-UEV binding interface, high-throughput screening Thermofluor and AlphaScreen miniaturized assays have been developed. The screening of different compound and drug-repurposing libraries have allowed the identification of set of small-molecule ligands that bind TSG101-UEV with IC50 values in the low micromolar range. High-content bimolecular complementation assays in human cells, Ebola and Marburg virus-like particle assays and antiviral activity assays against VSV and HIV-1 validated some of these compounds as broad-spectrum inhibitors TSG101-UEV/Late domain interactions. These results show that targeting TSG101-UEV interactions is a promising strategy toward the development of host-oriented, broad-spectrum antivirals.

1.- PTAP viral Late domains are recognized by TSG101-UEV with low binding affinities and favorable binding enthalpies, determined by water-mediated interactions

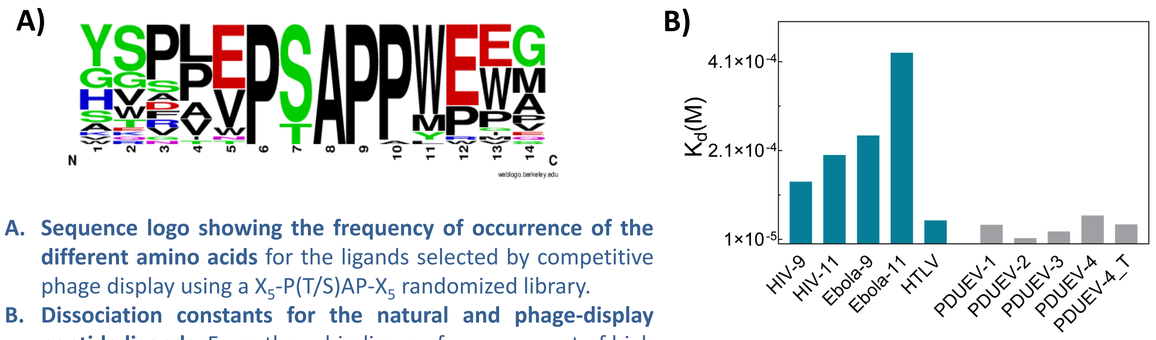


A. **Isothermal titration calorimetry**. Upper panel: Ebola titration (black line) and dilution experiment (red line). Lower panel: binding isotherm (black) and best fit to a one set of sites model (red).

B. **Persistent water-mediated interactions revealed by X-Ray structures and Molecular Dynamics simulations** are determinant for the observed binding energetics.

C. **Binding energetics of Late domain peptides to TSG101-UEV.** PTAP recognition is driven by markedly favorable binding enthalpies opposed by unfavorable entropic contributions.

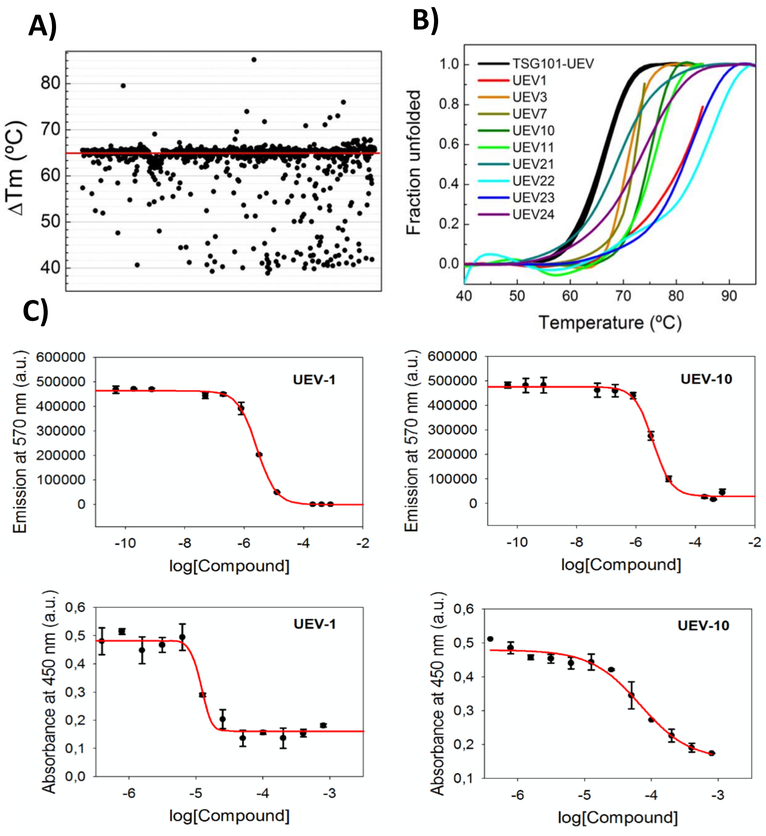
2.- Phage display shows that higher binding affinity is attainable for small peptide ligands of TSG101-UEV



A. **Sequence logo showing the frequency of occurrence of the different amino acids** for the ligands selected by competitive phage display using a X_5-P(T/S)AP-X_5 randomized library.

B. **Dissociation constants for the natural and phage-display peptide ligands.** From these binding preferences, a set of high affinity ligands were derived showing one order of magnitude improvement in binding affinity as measured by ITC.

3.- The TSG101-UEV binding interface is druggable: TSG101-UEV/Late domain interactions can be disrupted by small drug-like molecules

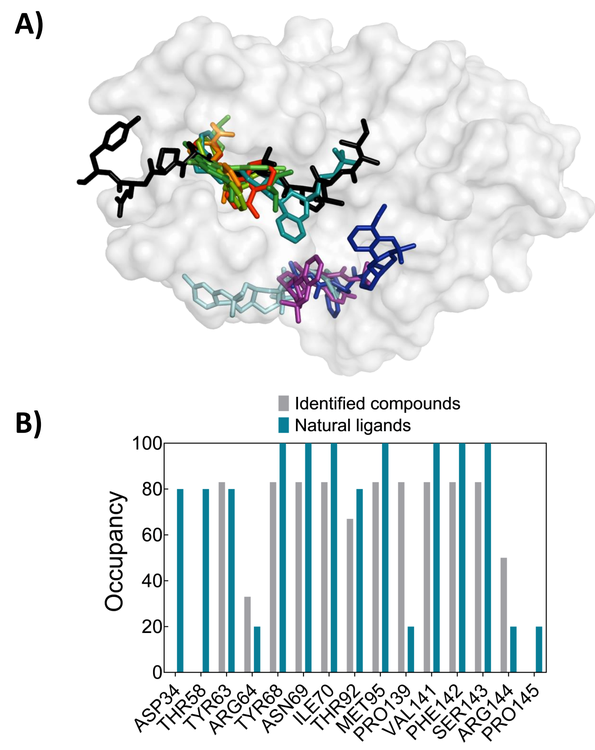


A. **Primary high-throughput screening** of the NCI Diversity Set IV and the Natural Products Set III libraries using differential scanning fluorimetry (DSF) to identify small-molecule binders of TSG101-UEV.

B. **Normalized DSF profiles for TSG101-UEV** free (black lines) and in the presence of small-molecule compounds (colored lines) inducing changes in Tm < 1ºC.

C. **Validation by orthogonal secondary screening of positives.** Dose-response curves obtained by AlphaScreen (upper panels) and ELISA (lower panels) competition assays against a pre-formed TSG101-UEV/HIV-p6 complex. Symbols represent the average of three independent replicas and error bars show the corresponding standard deviations. **These experiments confirm that the identified ligands disrupt this interaction with IC50 values in the micromolar range.**

4.- The small-molecule inhibitors and the Late-domain peptide ligands interact with the same binding site residues in TSG101-UEV

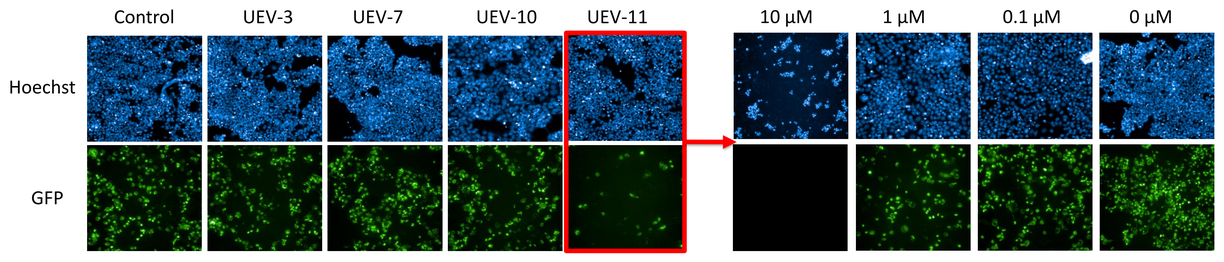


A. **Lowest energy poses obtained for the docking of the small-molecule inhibitors of viral Late domain interaction on the TSG101-UEV crystal structure (4EJE).** The validated positives were docked onto the structure of the TSG101-UEV domain extracted from the Ebola complex (white surface). The lowest energy pose for the different inhibitors are shown as colored sticks. As a reference, the Ebola Late domain peptide is shown in black.

B. **TSG101-UEV residues interacting with peptide and small-molecule ligands**. Shown is the frequency which TSG101-UEV residues were found to interact with the Late domain peptides (experimental crystal structures: blue bars) and with the small-molecule inhibitors (structural models from the docking study: grey bars).

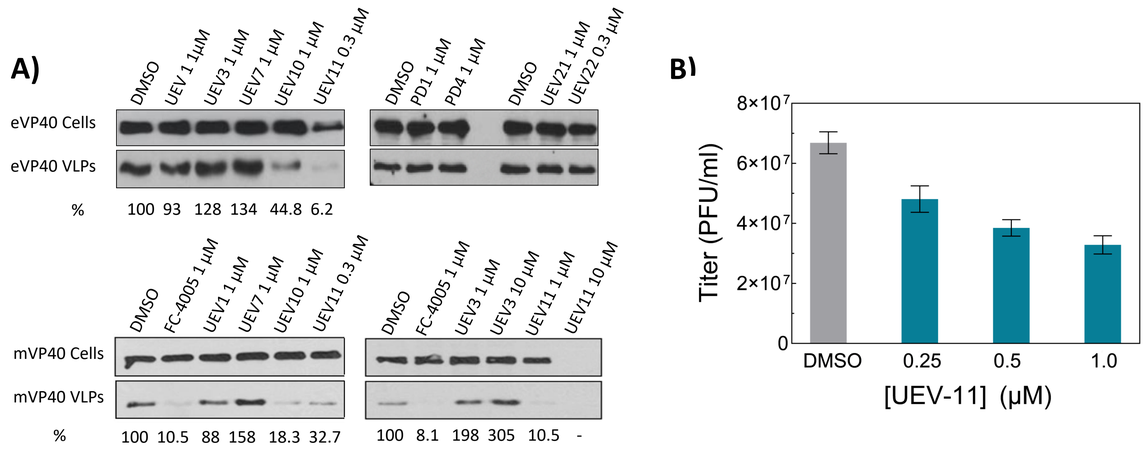
With the exception of a set of related compounds that are found to bind with lower energy to a second binding region, most small-molecule inhibitors bind preferentially to the Late-domain binding site, mimicking the interactions established by the conserved PTAP core motif.

5.- Small-molecule inhibitors can block the interaction between full-length TSG101-UEV and Ebola-VP40 in a cellular context



Cellular Images of the Bimolecular Fluorescence Complementation (BiFC) Cellular Assays. Green Fluorescence Protein (GFP) signal is produced upon interaction between TSG101 and Ebola-VP40. The 4 most promising compounds were tested at 10 μM concentration. UEV-11 was found to block the interaction between the full-length proteins at 0.1-1 μM without significant toxicity.

6.- Small-molecule inhibitors of TSG101-UEV/Late domain interactions can block particle egress for different viruses



A. **Virus-Like Particle assays (VLPs) performed with Ebola- and Marburg-VP40** in the presence of different inhibitors. At concentrations below 10 μM , UEV-11 shows inhibitory activity of Ebola and Marburg budding without toxicity.

B. **Vesicular Stomatitis Virus (VSV) proliferation assays**. UEV-11 shows dose-dependent inhibitory activity of viral replication against live VSV.

Conclusion: The multidisciplinary approach presented here, combining biophysical characterization, phage display and High-Throughput screening methodologies has allowed the identification of novel compounds blocking the interactions between TSG101-UEV and viral Late domains and disrupting budding and viral proliferation in several encapsulated virus, of interest as potential novel broad-spectrum antivirals.

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