Identification of ALIX-V Domain Ligands of Interest as Broad-Spectrum Antivirals using phage display

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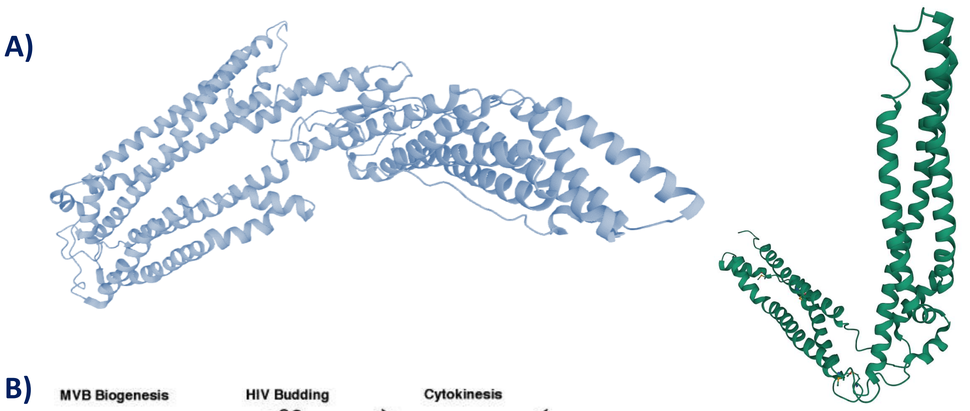
ABSTRACT.- Recognition of the LYPX(n)L viral late domains by the V domain of programmed death interacting protein 6 (AIP6) or ALIX, is essential for the budding of many enveloped viruses, such as HIV, Ebola and EIAV. Blocking these interactions is a promising strategy for developing broad-spectrum antivirals. In this work, we tackle the study of the binding preferences of ALIX-V and the identification of high-affinity peptide sequences through the design and screening of randomized peptide libraries by phage display. Our results reveal a strong preference for n = 3 in high affinity ligands of the YPX(n)L type for ALIX-V. The binding of selected high-affinity sequences has been validated by MicroScale Thermophoresis, showing that the selected peptides exhibit competitive binding with natural ligands and providing valuable key elements for the search and determination of a high-affinity binding.

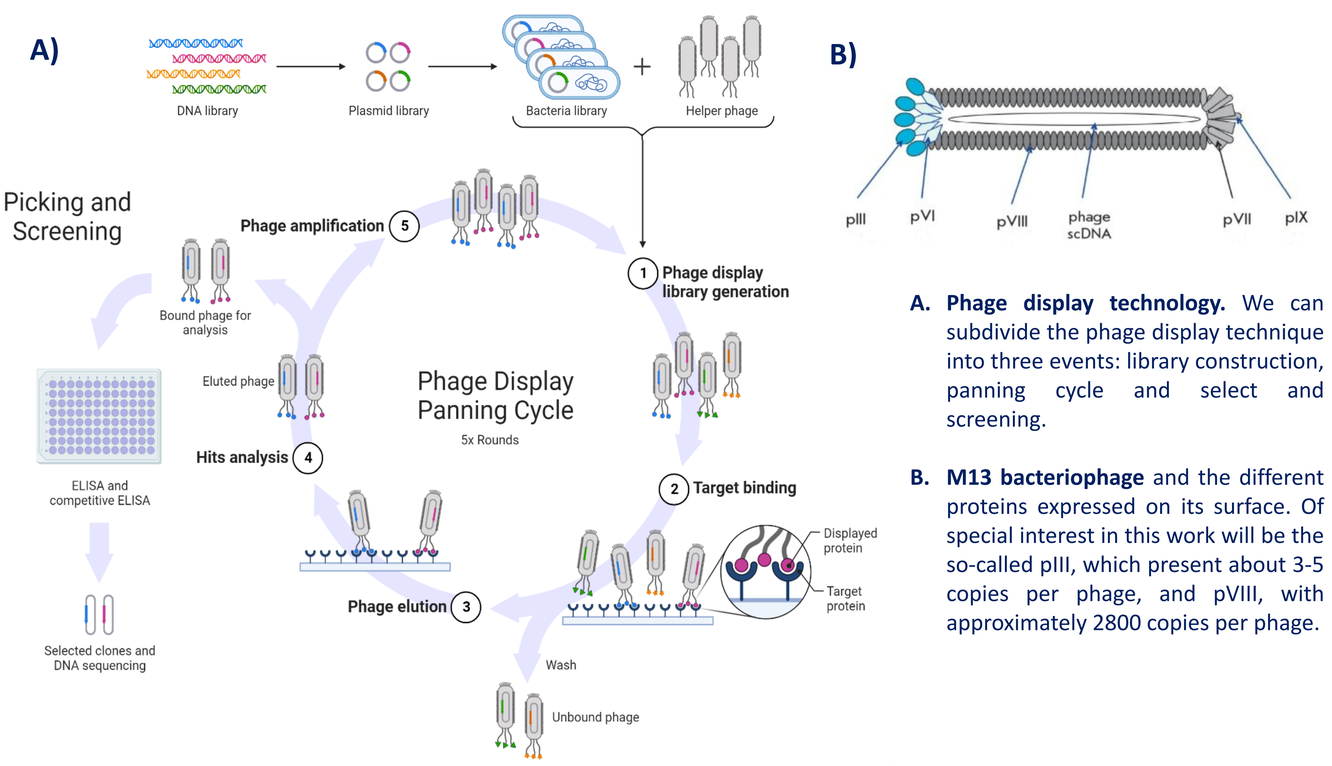
1.- The importance of the search for high-affinity peptide ligands of the human protein ALIX-V



A. **Structure and overall fold of ALIX** in blue **and ALIX domain V** in green.

B. **ESCRT pathway functions**, in which ALIX protein participates. ALIX, among others, can be recruited by adapter proteins (red) in terminal membrane fission events of MVB biogenesis, virus budding, and cytokinesis.

2.- Experimental protocol of phage display in search for peptide ligands



A. **Phage display technology.** We can subdivide the phage display technique into three events: library construction, panning cycle and select and screening.

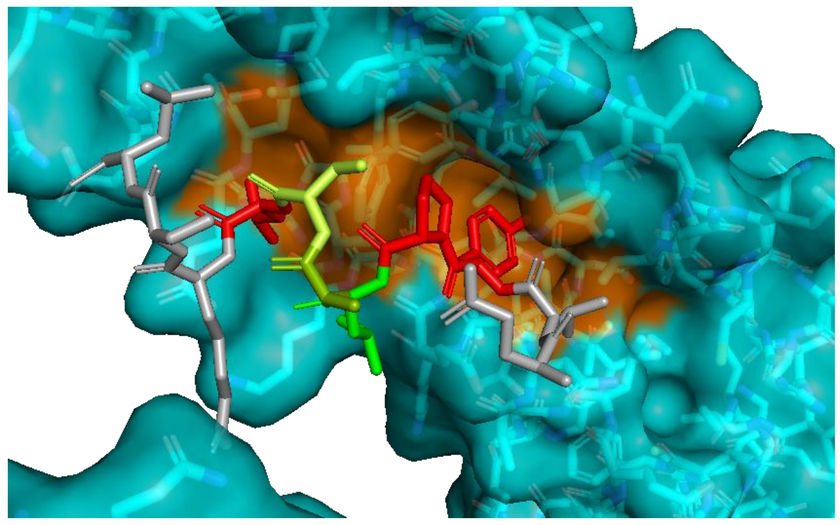
B. **M13 bacteriophage** and the different proteins expressed on its surface. Of special interest in this work will be the so-called pIII, which present about 3-5 copies per phage, and pVIII, with approximately 2800 copies per phage.

3.- Phage display reveals a clear preference for n=3 in $YPX_{(n)}L$ ALIX-V ligands

A. **Sequence logo showing the frequency of occurrence of the different amino acids** for the ligands selected by competitive phage display using a X_{12} randomized library in pVIII. A marked preference for Y and W, in positions 2 and 4, is observed. Also, the first position would correspond to an aliphatic amino acid, and in the core motif (6-7 positions), L seems to be selected.

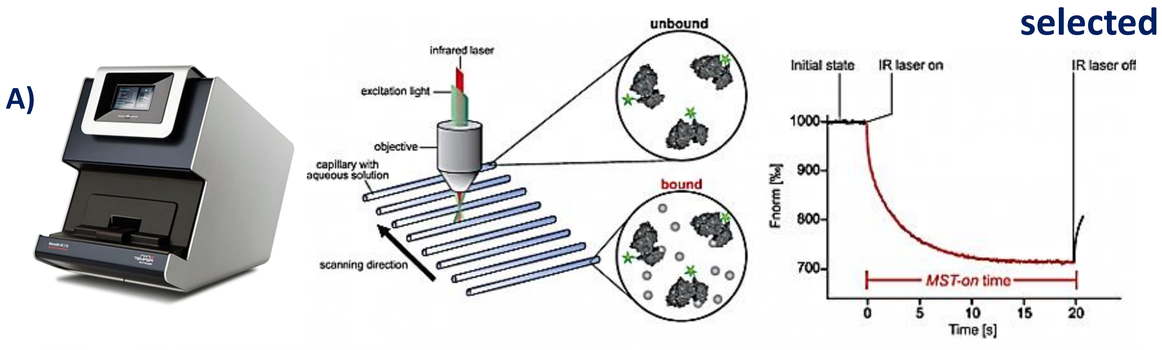
B. **Sequence logo showing the frequency of occurrence of the different amino acids** for the ligands selected by competitive phage display using a (I/F)YPWX_6 (left panel) and a (I/F)YPWX_2LX_3 (right panel) randomized libraries. In both cases a strong preference for I in the first position, two negatively charged residues bridging the YP and L-residues in the core motif is observed.

C. **Sequence logo showing the frequency of occurrence of the different amino acids** for the ligands selected by competitive phage display using a $X_3YPX_2LX_4$ randomized library. The results seem to confirm A, B. In addition to a marked preference for L residues in the central motif, this amino acid also appears to be selected in the final position.

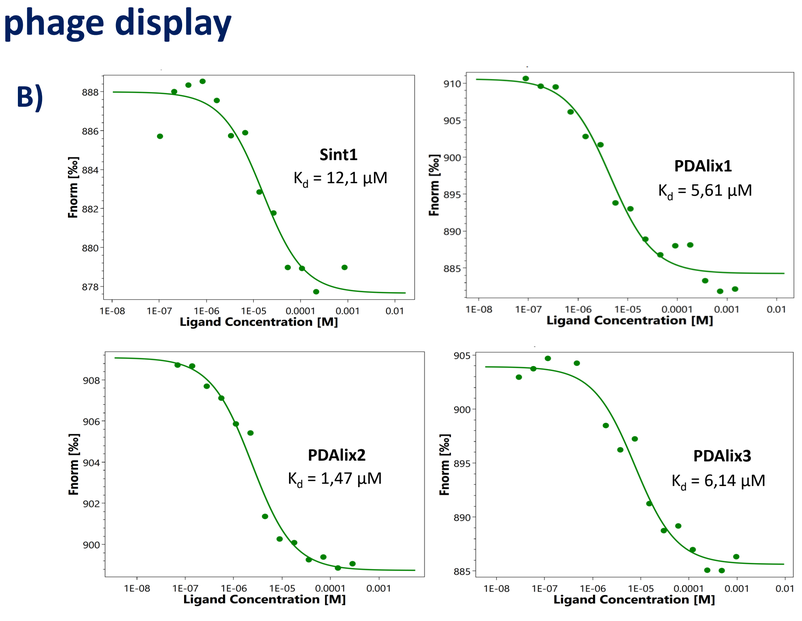


D. **Crystal structure of the ALIX-V domain in complex with the HIV-1 YPLTSL Late domain sequence conforming to the preferred YPx_3L pattern (PDB. Code: 2R02).** The ALIX-V domain is shown in a surface representation. The hydrophobic binding site is highlighted in orange. The Late domain peptide is shown as sticks, where the YP and L residues in the core motif packing against the ALIX-V hydrophobic pockets are shown in red and the connecting residues in green.

4.- MST experiments confirm binding and establish similar dissociation constants for the natural cellular and viral peptide ligands and peptides selected by phage display



A. **Microscale thermophoresis.** MST is a method to quantify biomolecular interactions. It measures the movement of molecules along microscopic temperature gradients in capillaries and detects changes in their hydration layer, charge, or size. When performing an MST experiment, an IR laser induces a microscopic temperature gradient, and the movement of molecules is detected and quantified using covalently bound dyes, fluorescent fusion proteins, or intrinsic tryptophan fluorescence, as shown in the top center figure.



B. **Dissociation constants measured by MST for the natural and phage-display peptide ligands.** Binding affinity of the V domain of ALIX in interaction to the human ligand Sint1 (Ac-MSLYPSLEDLKV-NH_2, extracted from human protein syntenin-1), and the peptides determined by phage display PDAlix1, PDAlix2, PDAlix3.

Conclusion: Phage display is a highly potent methodology to optimize small peptide-protein interactions. In this research, we identified three peptides with specific binding capacity to ALIX-V, which represents an advance in the search for new broad-spectrum antivirals that allow the interruption of viral budding and the proliferation of retroviruses.

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