

# Peptide ligands targeting the vesicular stomatitis virus G (VSV-G) protein for the affinity purification of lentivirus particles

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## Abstract

The recent uptick in the approval of *ex vivo* cell therapies highlight the relevance of Lentivirus (LV) as an enabling viral vector of modern medicine. As labile biologics, however, LVs pose critical challenges to industrial biomanufacturing. In particular, LV purification – currently reliant on filtration and anion-exchange or size-exclusion chromatography – suffers from long process times and low yield of transducing particles, which translate in high waiting time and cost to patients. Seeking to improve LV downstream processing, this study introduces peptides targeting the enveloped protein Vesicular stomatitis virus G (VSV-G) to serve as affinity ligands for the chromatographic purification of LV particles. An ensemble of candidate ligands was initially discovered by implementing a dual-fluorescence screening technology and a targeted *in silico* approach designed to identify sequences with high selectivity and tunable affinity. The selected peptides were conjugated on Poros resin and their LV binding-and-release performance was optimized by adjusting the flow rate, composition, and pH of the chromatographic buffers. Ligands GKEAAFAA and SRAFGVDARD were selected for their high product yield (50-60% of viral genomes; 40-50% of HT1080 cell-transducing particles) upon elution in PIPES buffer with 0.65 M NaCl at pH 7.4. The peptide-based adsorbents also presented remarkable values of binding capacity (up to  $3 \cdot 10^9$  TU per mL of resin at the residence time of 1 min) and clearance of host cell proteins (up to 220-fold reduction of HEK293 HCPs). Additionally, GKEAAFAA demonstrated high resistance to caustic cleaning-in-place (0.5 M NaOH, 30 min) with no observable loss in product yield and quality.

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