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Novel viral peptide-based tandemized carriers: Structure prediction and *in vitro* evaluation

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Introduction

Previously we have shown the efficacy of a modified synthetic peptide (RF) derived from the Herpes simplex virus-1 gD glycoprotein (^{228}QRTVAVYSLRIAGFHG^{243}) (Fig. 1) ([1] in internalization into epithelial or neural cells. Tuftsin derivatives (OT5(P), OT5) can deliver cargo into different cells via neuropilin receptors [2-4]. Positively charged penetratin, the most studied cell-penetrating peptide (Pen), can rapidly enter cells.
We aimed to modify the RF peptide's cellular uptake rate and intracellular localization by tandemizing it with tuftsin or penetratin derivatives (Table 1, Fig. 2).
Internalization was evaluated on VERO E6 non-human primate kidney epithelial and A431 human skin squamous carcinoma cells.

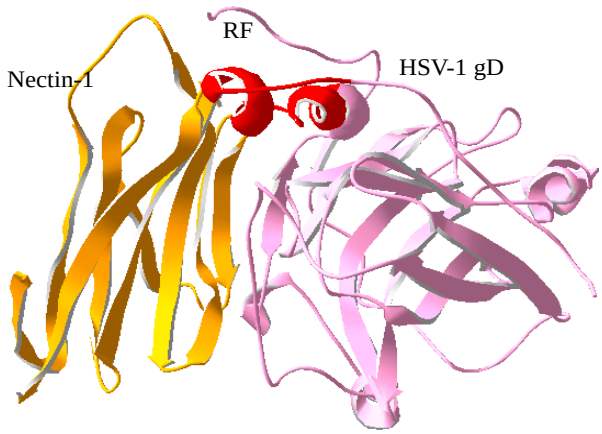

Figure 1. Secondary structure of HSV-1 gD–nectin-1 cell adhesion complex (PDB: 3U82) for virus entry. HSV gD 228-243 region show cellular entry ability as a synthetic peptide (Cf-RF).

Results and Discussions

Internalization of synthetic Cf-labeled peptides and tandems was measured by BD LSR II flow cytometer. All Cf-peptides containing the RF sequence internalize in a concentration-dependent manner (UC_{50} concentration value for Cf-positivity in 50% of the live cells: 0.1 – 5 μM) (Figure 2, Table 1). No cytotoxicity was detected (Figure 2, inserts). According to confocal laser scanning microscopy Cf-RF showed cytoplasmic localization; its tandemization with the tuftsin derivative resulted in compartmentalized localization to different degrees (data not shown).

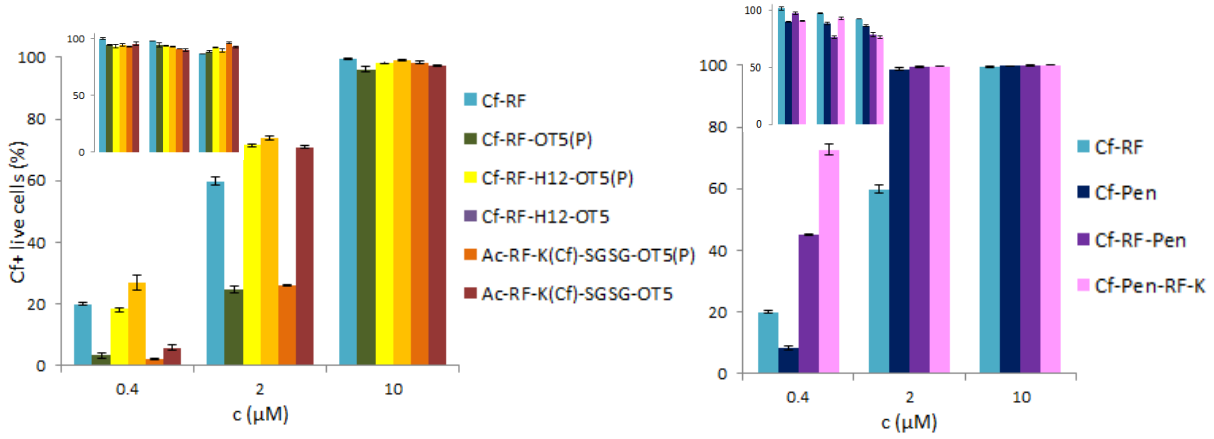

Figure 2. Internalization of Cf-peptides into A431 epithelial cells (B), represented by Cf+ live cell percentage. Inserts: relative viability (%).

Table 1. Analytical data, and internalization of Cf-labeled peptides characterized by UC_{50} (concentration value for Cf-positivity in 50% of the live cells)

Peptide code	*Mw, average*[a]		*UC_{50} (μM)*[b]	
	calc.	*meas.*	*A431*	*VERO E6*
Cf-RF	2132.36	2132.17	1.34	4.72
Cf-RF-OT5(P)	2712.10	2710.81	3.54	1.36
Cf-RF-H12-OT5(P)	3859.31	3857.33	1.04	1.08
Cf-RF-H12-OT5	3791.23	3790.98	0.88	2.37
Ac-RF-K(Cf)-SGSG-OT5(P)	3170.49	3169.44	3.41	0.51
Ac-RF-K(Cf)-SGSG-OT5	3102.41	3101.13	1.19	1.23
Cf-Pen	2605.06	2603.08	0.84	0.10
Cf-RF-Pen	4361.11	4361.42	0.46	0.60
Cf-Pen-RF-K	4489.28	4489.82	0.24	0.54

[a] *ESI-MS (Bruker Amazon SL ESI Mass Spectrometer, Bremen, Germany)*
[b] *Concentration value for Cf-positivity in 50% of the live cells (BD LSR II flow cytometer, data evaluation: FACSDiva 5.0 software.*

The secondary structure of tandem peptides (Pep-Fold 3) [5] depends on the linker moiety. The H12 rigid spacer may help the RF region to adopt its native helical structure, thus helping internalization in VERO E6 cells.
Penetratin derivatives and tandems internalize the most effectively; in A431 cells, nectin-binding HSV gD RF peptide enhances the internalization. Different spacers and tuftsin vs. tuftsin agonists in the tandemized peptides showed opposite orders of internalization in A431 and VERO E6 cells. Internalization into VERO E6 cells is favored by the presence of linker, preferably a shorter, more flexible one. On the other hand, in A431 cells the rigid linker proved more effective.

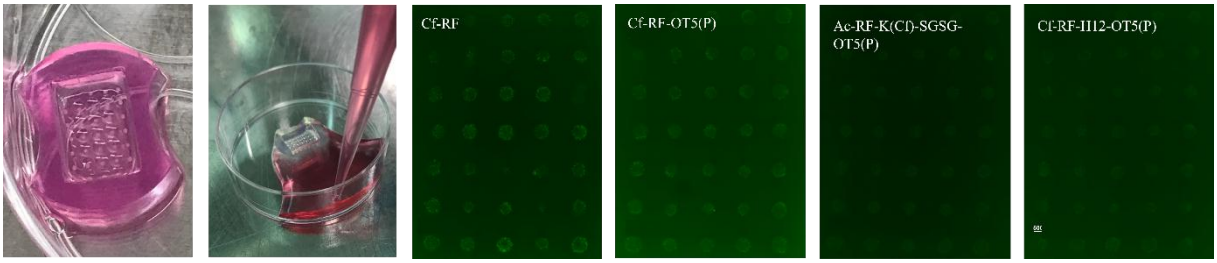

Figure 3. Penetration studies on agarose dish-based A431 spheroids, treatment applying 12.5 μM Cf-peptides, 3h.

The tandem peptides' penetration (with low UC_{50} values) was evaluated on spheroids as simple *in vitro* tissue-mimicking milieu, and all tandems demonstrated fair penetration ability (Figure 3).
These peptides are promising carrier candidates with variable intracellular trafficking depending on their sequence.

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