Supplementary Information

Self-assembly stability compromises the efficacy of tryptophan-containing designed anti-measles virus peptides

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HRC-L454W peptides secondary structure

HRC-L454W peptides secondary structure was studied using circular dichroism (CD). Peptide samples were prepared in 2% TFE (v/v) in 10 mM phosphate, 150 mM NaF, pH 7.4 buffer. CD spectra of the peptides at 10 μM were acquired at 25ºC, between 190 and 260 nm. Each final spectrum corresponds to the averaged accumulation of 10 scans. Spectra were plotted using mean molar residue ellipticity ([θ]) values [1]:

$$[\theta] = \frac{\theta}{aa \times l \times c} \qquad \text{(S1)}$$

in which θ is the ellipticity, *aa* the number of amino acid residues of the peptide sequence, *l* is the cuvette path length, and *c* is the molar concentration. [θ] values were corrected for the buffer background noise. Two independent replicates were performed for each sample.

The helix content (X_H) of each peptide was calculated using the equation:

$$X_H = \frac{[\theta]_{222}}{[X_H^{\infty}\left(1-\frac{k}{n}\right)]\times 100} \quad \text{(S2)}$$

in which $[\theta]_{222}$ is the mean residue molar ellipticity at 222 nm, X_H^{∞} is the reference value for a helix of infinite length, *n* the number of residues per helix, and *k* a wavelength-dependent constant. X_H^{∞} and *k* values, -39.5 deg.cm^2.dmol^{-1} and 2.57, respectively, were obtained from computed CD and optical rotatory dispersion (ORD) of a helix of infinite length [2].
Also, the ratio $[\theta]_{222}/[\theta]_{208}$ was obtained. Parallel $n\pi^*$ and $\pi\pi^*$ α-helix backbone excitation bands occur at the 222 and 208 nm, respectively, being the $[\theta]_{222}/[\theta]_{208}$ ratio used to evaluate the α-helices flexibility [3, 4].

CD analysis was used to determine the peptides' secondary structure and evaluate the solvent-exposition and looseness of the peptide chains within the nanoparticles (Figure S1 and Table S1). HRC4, -7 and -8-L454W displayed the lowest helical content. Additionally, these peptides revealed higher $[\theta]_{222}/[\theta]_{208}$ values when compared to those observed for the respective mono-conjugated monomeric HRC2 and -5-L454W peptides. These data suggest a reduced hydrophobic shielding of the peptide chains within the HRC4, -7 and -8-L454W nanoparticles, leading to a loss on their helical properties.

Bibliography

1. Kelly SM, Jess TJ, Price NC (2005) How to study proteins by circular dichroism. Biochim Biophys Acta - Proteins Proteomics 1751:119–139
2. Chen YH, Yang JT, Chau KH (1974) Determination of the helix and beta form of proteins in aqueous solution by circular dichroism. Biochemistry 13:3350–3359
3. Greenfield N, Fasman GD (1969) Computed Circular Dichroism Spectra for the Evaluation of Protein Conformation. Biochemistry 8:4108–4116
4. Cooper TM, Woody RW (1990) The effect of conformation on the CD of interacting helices: A theoretical study of tropomyosin. Biopolymers 30:657–676

Figures

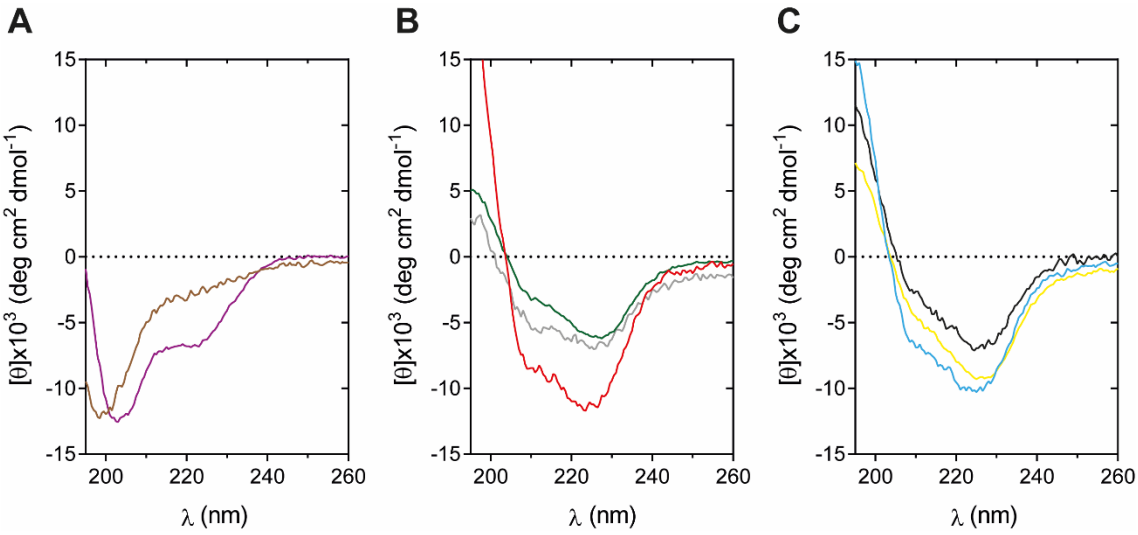

Figure S1

Figures Legend:

Figure S1 - Representative CD spectra of each non-conjugated (A), Chol-conjugated (B) and Toc-conjugated (C) HRC-L454W peptide samples (HRC1-L454W, brown; HRC2-L454W, red; HRC3-L454W, purple; HRC4-L454W, green; HRC5-L454W, blue; HRC6-L454W, yellow; HRC7-L454W, grey; HRC8-L454W, black).

Tables

Table S1 – HRC-L454W α-helix parameters. α-helical content was calculated through the equation S2.

	X_H (%)	$[\theta]_{222}/[\theta]_{208}$
HRC2-L454W	26,62 ± 5,16	1,549 ± 0,054
HRC4-L454W	12,40 ± 3,45	1,835 ± 0,517
HRC5-L454W	29,964 ± 4,562	1,674 ± 0,090
HRC6-L454W	22,3 ± 0,06	2,276 ± 0,214
HRC7-L454W	16,706 ± 0,806	1,702 ± 0,188
HRC8-L454W	16,36 ± 1,32	2,550 ± 0,325