

SUPPORT INFORMATION

**Amphipathic Design Dictates Self-Assembly, Cytotoxicity and
Cell Uptake of Arginine-rich Surfactant-like Peptides**

Lucas R. Mello,^a Rodrigo B. Aguiar,^a Renata Y. Yamada,^a Jane Z. Moraes,^a Ian W. Hamley,^b Wendel A. Alves,^c Mehedi Reza,^d Janne Ruokolainen^d and Emerson R. Silva^{a*}

^aDepartamento de Biofísica, Universidade Federal de São Paulo, 04044-020, São Paulo, Brazil; ^bDepartment of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, UK; ^cCentro de Ciências Naturais e Humanas, Universidade Federal do ABC, 09210-580, Santo André, Brazil; ^dDepartment of Applied Physics, Aalto University School of Science, P.O. Box 15100, FI-00076 Aalto, Finland.

- ⇒ Chromatography and mass spectrometry data;
- ⇒ CD experiments;
- ⇒ Table with SAXS fitting parameters;
- ⇒ AFM topography images;
- ⇒ Cell fluorescence imaging.

CHROMATOGRAPHY AND MASS SPECTROSCOPY DATA

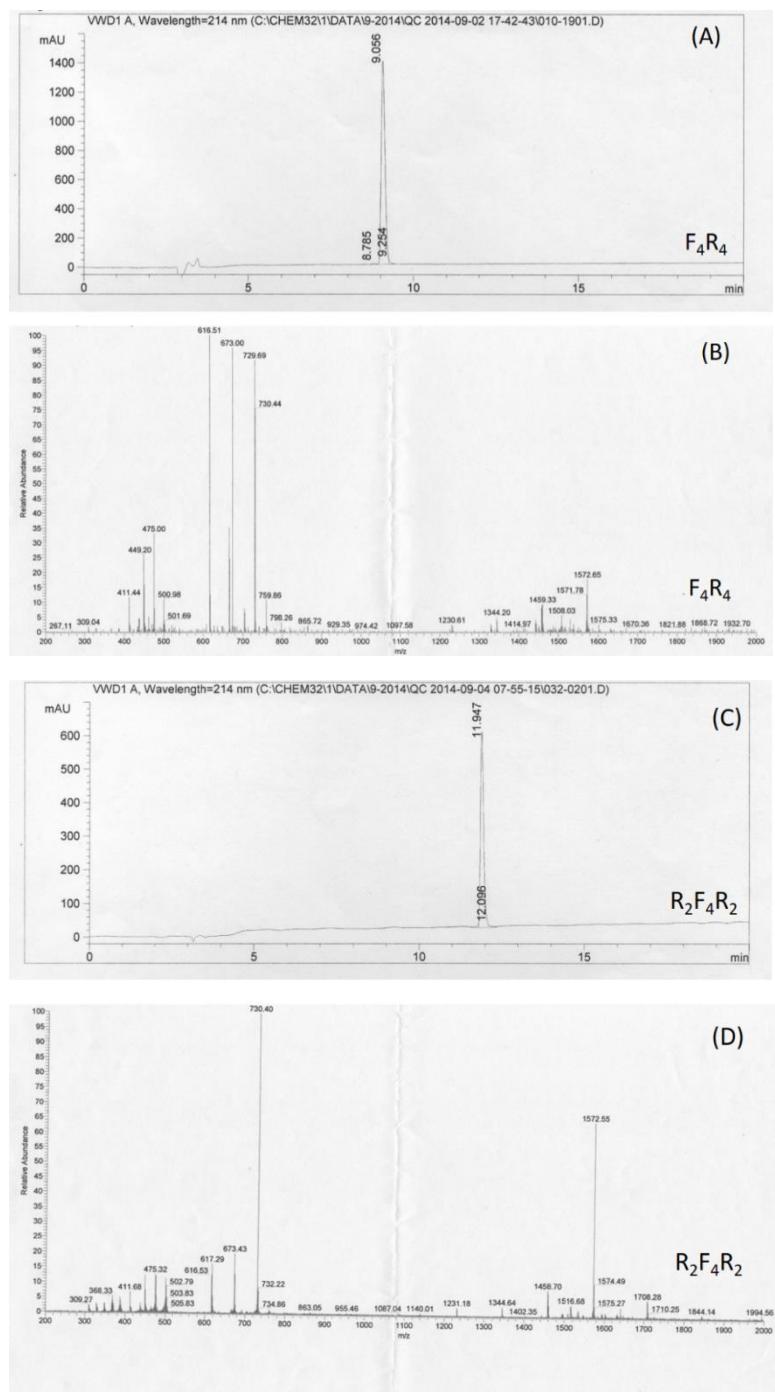


Fig. S1. Chromatograms and mass spectra from diblock peptides (A and B) and from the bolaamphiphile variants (C and D).

CD EXPERIMENTS

Experimental procedure: CD data were obtained using a JASCO 810 instrument, from samples prepared at 50 mg/ml, carefully placed between the windows of demountable quartz cuvettes with 0.1 mm pathlength. A total of 6 accumulations were obtained, in the range 190-250 nm, 1 nm per step, at 50 nm/min. All curves were background subtracted and curves were smoothed with FFT filters (window = 5 points) to eliminate stochastic noise

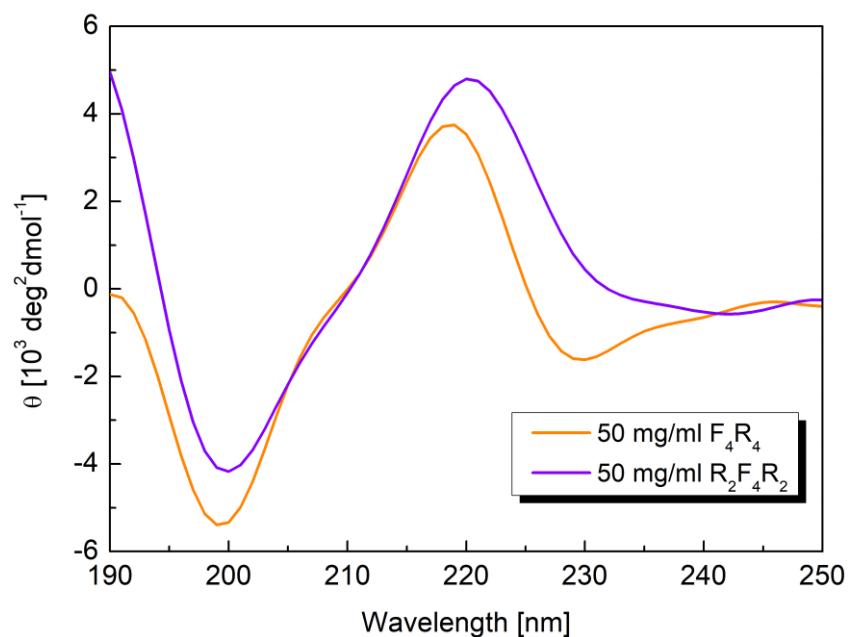


Fig. S2. Circular dichroism spectra from solutions containing amphipathic peptides at 50 mg/ml.

SAXS FITTING PARAMETERS

Table S1. Summary of structural parameters arising from model fits of SAXS data shown in Figure 3. R_g = radius of gyration of mass fractal objects, R_c = inner radius of sphere or cylinder shells, ΔR = shell thickness, L = length of rod-like objects, σ_H = standard deviation of Gaussian function describing bilayer polar heads, σ_C = standard deviation of Gaussians describing bilayer hydrophobic core, t = separation between centers of Gaussians used for describing polar headgroups. Uncertainties associated to radii of polydisperse sphere/cylinder shells correspond to standard deviations of Gaussian distributions.

Peptide	Conc. (mg/ml)	Mass Fractal	Spherical Shell	Cylinder Shell	Gaussian Bilayer
F₄R₄	10	$R_g = 0.8$ nm $d = 2.7$	$R_C = 19.2$ ± 16.0 nm $\Delta R = 6.5$ nm	*****	*****
	50	$R_g = 0.7$ nm $d = 2.9$	*****	$R_C = 3.2$ ± 1.9 nm $\Delta R = 4.2$ nm	*****
	100	$R_g = 0.8$ nm $d = 2.7$	*****	$R_C = 2.0$ ± 1.7 nm $\Delta R = 4.2$ nm $L = 76.5$ nm	*****
R₂F₄R₂	10	$R_g = 0.8$ nm $d = 2.8$	*****	*****	$\sigma_H = 0.8$ nm $\sigma_C = 1.2$ nm $t = 2.9$ nm
	50	$R_g = 0.6$ nm $d = 2.8$	*****	*****	$\sigma_H = 0.5$ nm $\sigma_C = 1.2$ nm $t = 2.2$ nm
	100	$R_g = 0.7$ nm $d = 2.9$	*****	*****	$\sigma_H = 0.6$ nm $\sigma_C = 1.4$ nm $t = 3.3$ nm

AFM IMAGING FROM CONCENTRATED SOLUTIONS

Experimental procedure: AFM measurements from concentrated samples were performed using a Park NX10 instrument operating in tapping mode (LNNano, Campinas-Brazil). Droplets from peptide aqueous solutions were deposited onto the surface of freshly cleaved mica substrates and left to rest for about 5 minutes. The substrates were then rinsed with ultrapure water and left to dry overnight into desiccators. All measurements were carried out at room temperature with controlled humidity (< 3%), using 512×512 pixels, at scan rate of 0.3 Hz and cantilever frequency at ~ 190 kHz.

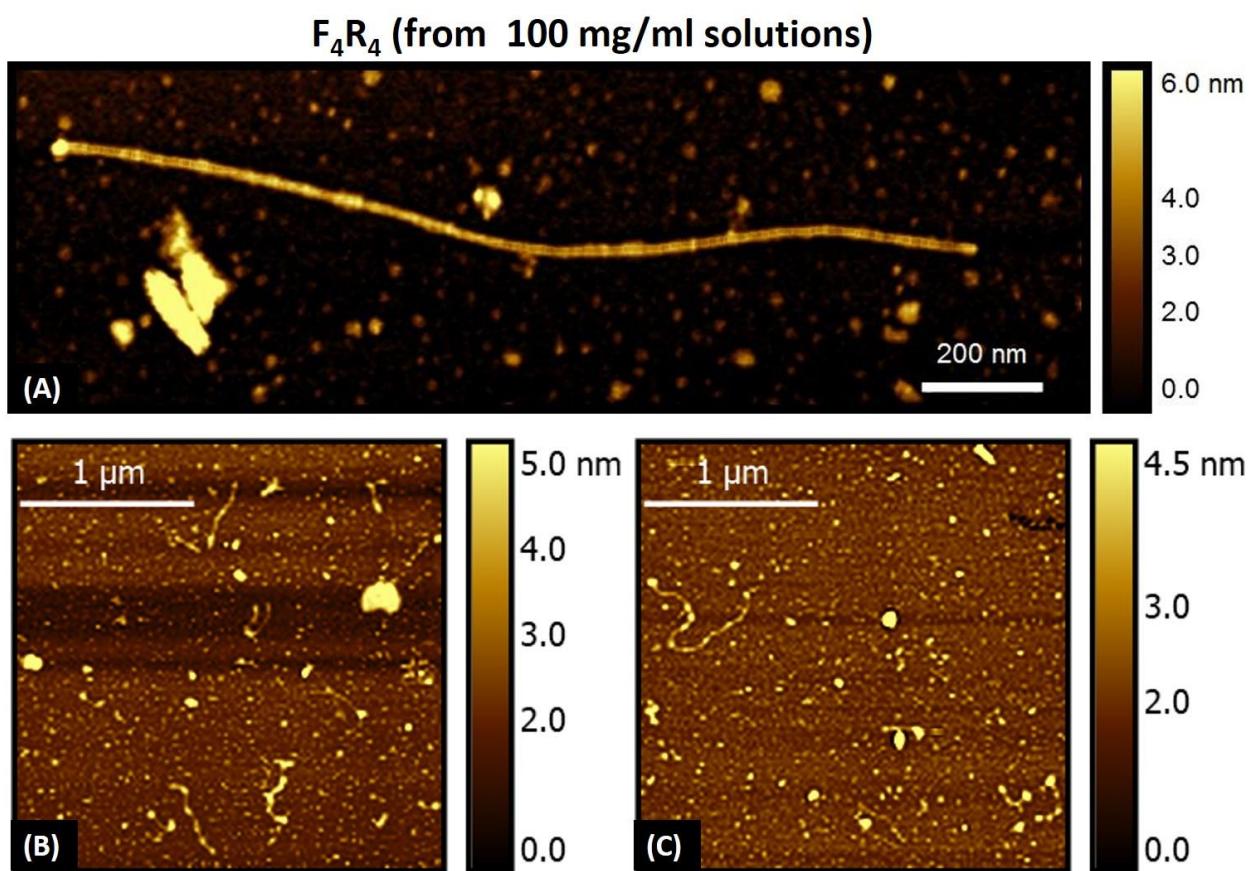


Fig. S3. Topography images from F_4R_4 nanoparticles obtained from solutions prepared at 100 mg/ml. The presence of worm-like assemblies is observed alongside globular aggregates.

$\text{R}_2\text{F}_4\text{R}_2$ (from 100 mg/ml solutions)

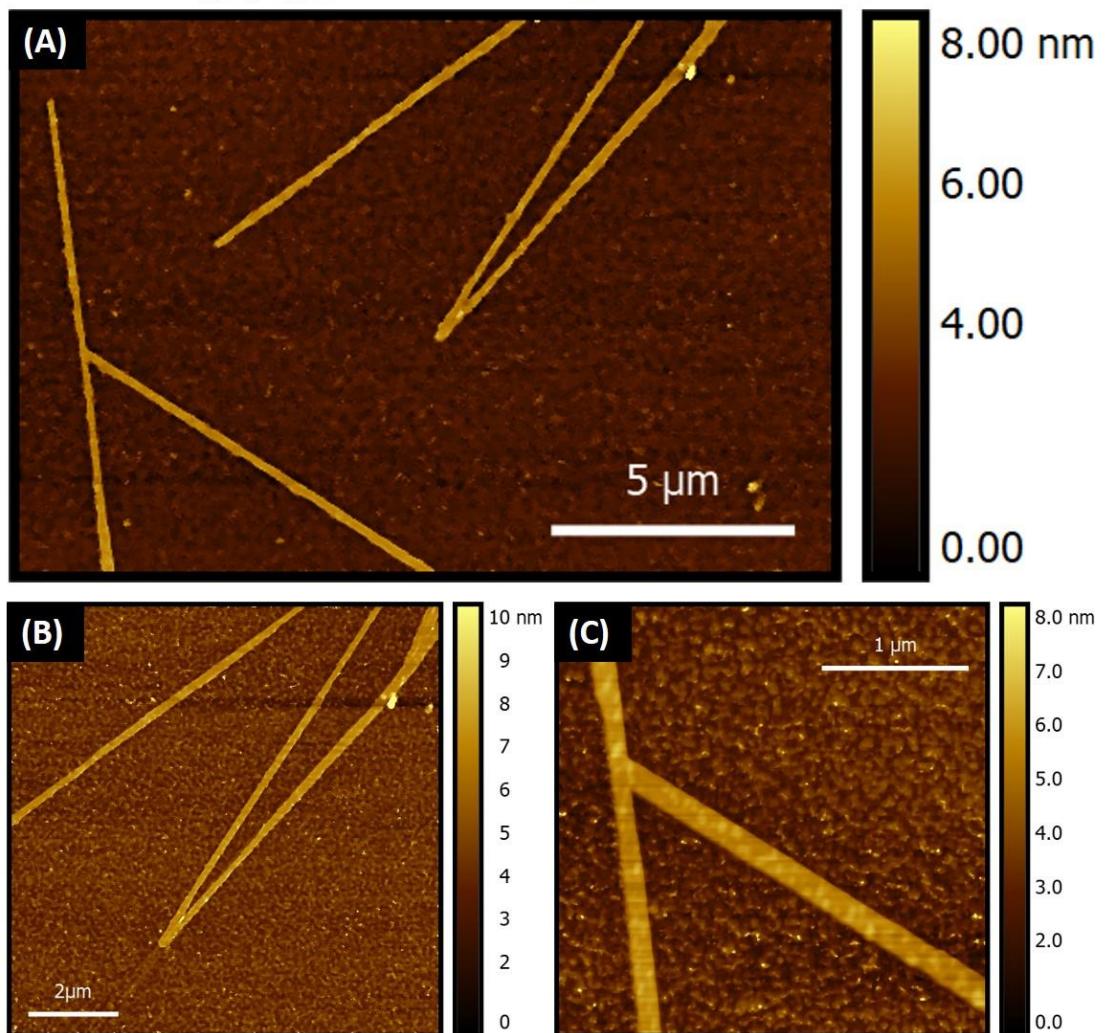


Fig. S4. Topography images from assemblies obtained from 100 mg/ml $\text{R}_2\text{F}_4\text{R}_2$ solutions. The extensive presence of interconnected tape-like (rigid) assemblies is observed across the sample.

AFM IMAGING FROM DILUTED SOLUTIONS

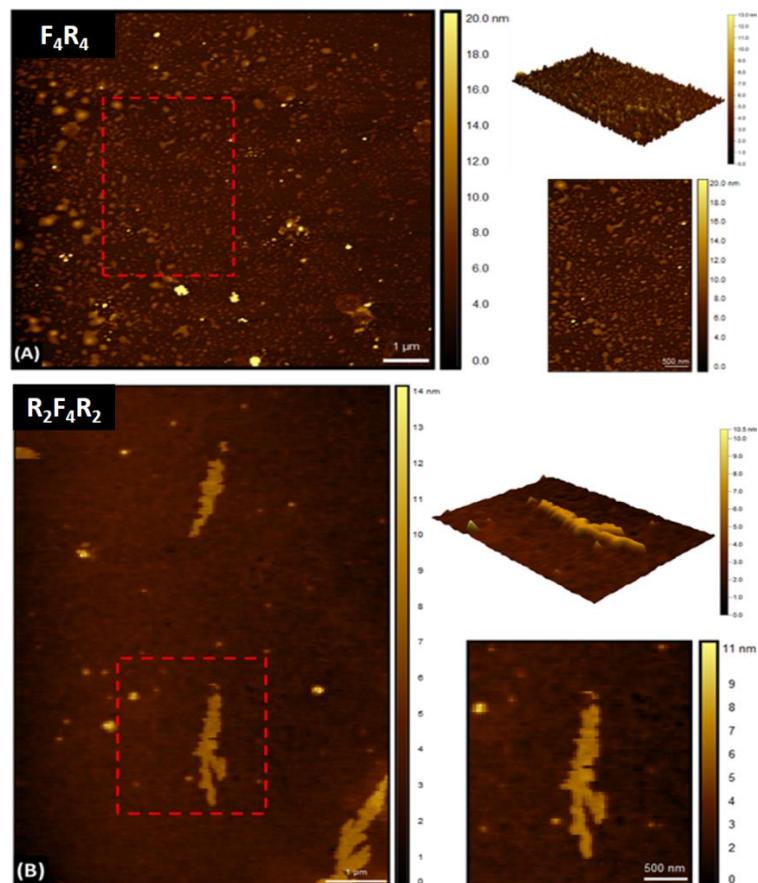


Fig. S5. AFM micrographs from dried solutions containing peptides at 1 mg/mL. Dashed squares indicate magnification zones and at the right upper corner of each image there is a 3D reconstruction of the samples. Globular aggregates from F₄R₄ samples have average diameter of 92 ± 61 nm ($n = 200$).

AFM IMAGING FROM BIOTIN-LABELED PEPTIDES

Experimental procedure: AFM measurements from biotin-labelled peptides peptides were performed on a Park XE7 instrument at Analitica LTDA (Sao Paulo, Brazil). The microscope operated in tapping mode, at scan rate of 0.3 Hz and tip frequencies near to 190 kHz. Samples from peptide solutions were prepared in the same manner described above (see Fig. S3 and S4). All measurements were carried out at room temperature. Data treatment was carried out using the software Gwyddion.

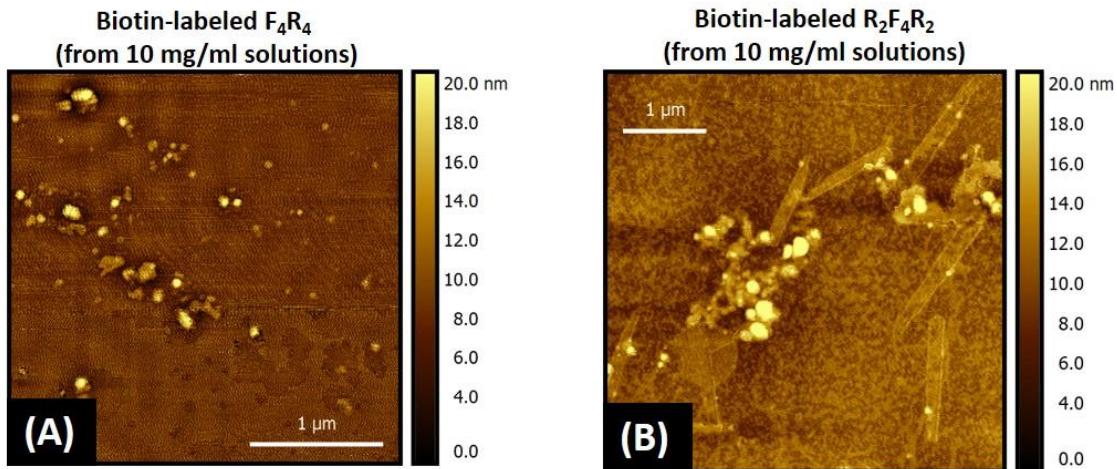


Fig. S6. AFM topography images from nanoparticles obtained from self-assembly peptides labelled with the biotin probe. Biotin-labelled F₄R₄ is found to self-assemble into globular particles, whereas the bolaamphiphilic R₂F₄R₂ appears organized into tape-like structures coexisting with round aggregates.

CELL FLUORESCENCE IMAGING

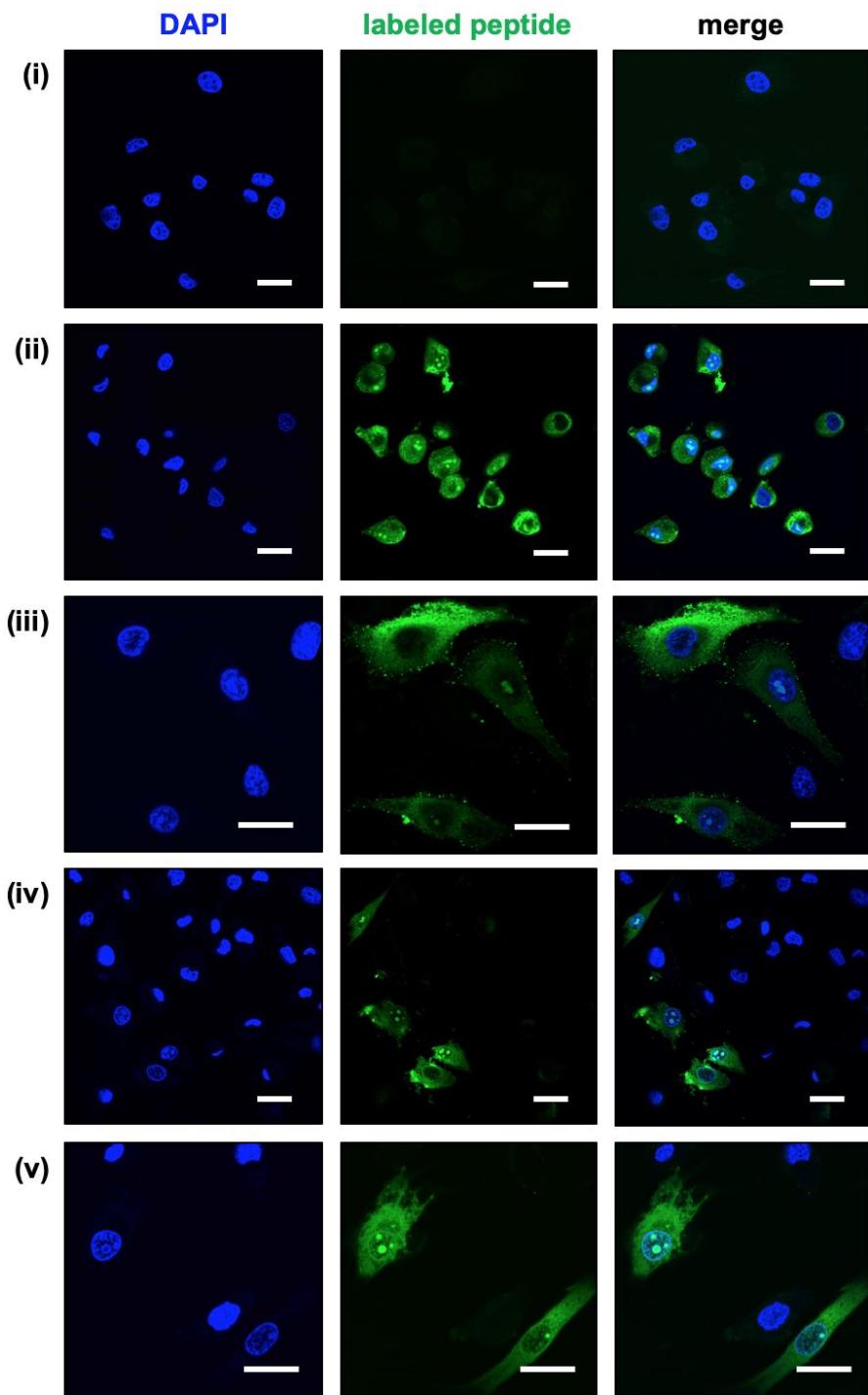


Fig. S7. Confocal fluorescence images used to construct the panels (i-v) provided in Fig. 5A (see main text). Images show the detection of DAPI (blue; left column) and the labeled peptide (green; middle column) on SK-Mel28 cells exposed for 30 min to R₄F₄ or R₂F₄R₂. The merged images are on the right column. DAPI stains nuclei and biotin-labeled peptides (green) were detected with Alexa Fluor® 488-conjugated streptavidin staining. Scale bar, 25 μ m.