

Supporting Information

Supramolecular Presentation of Bioinstructive Peptides on Soft Multilayered Nanobiomaterials Stimulates Neurite Outgrowth

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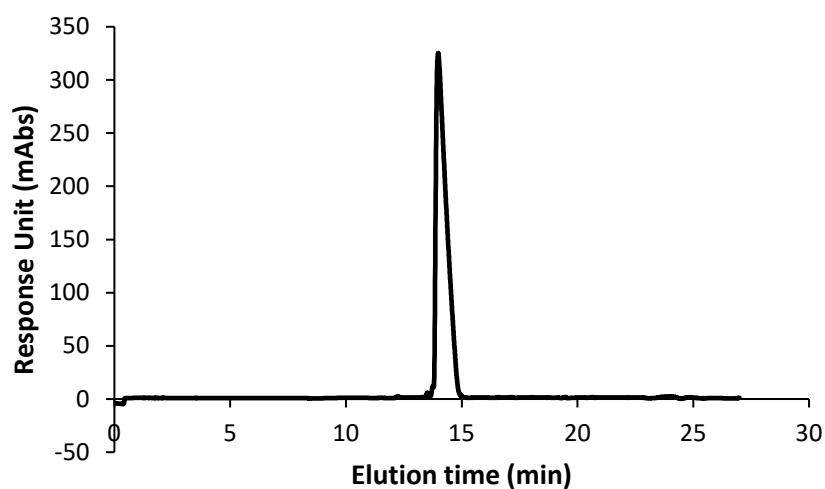
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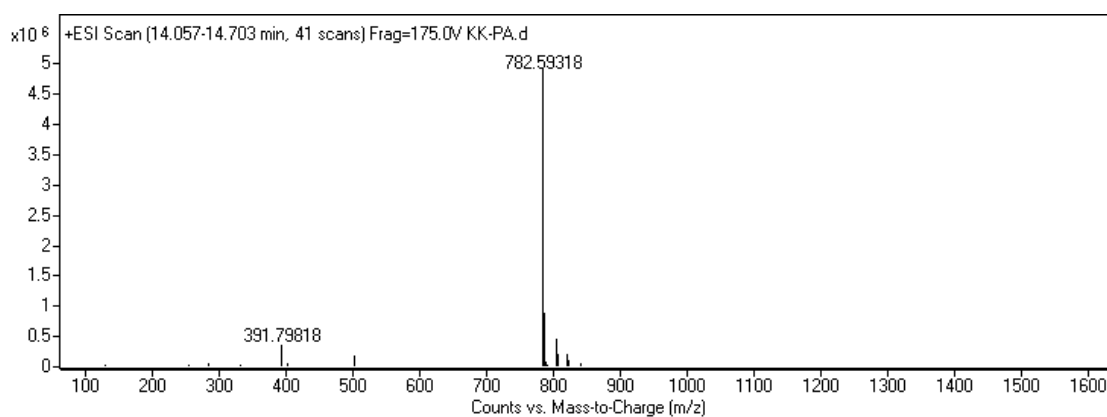
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(A)



(B)



(C)

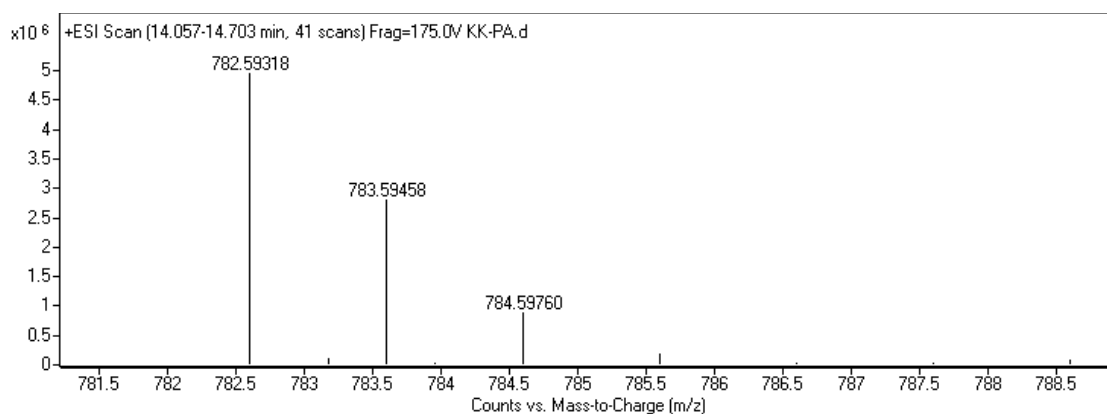
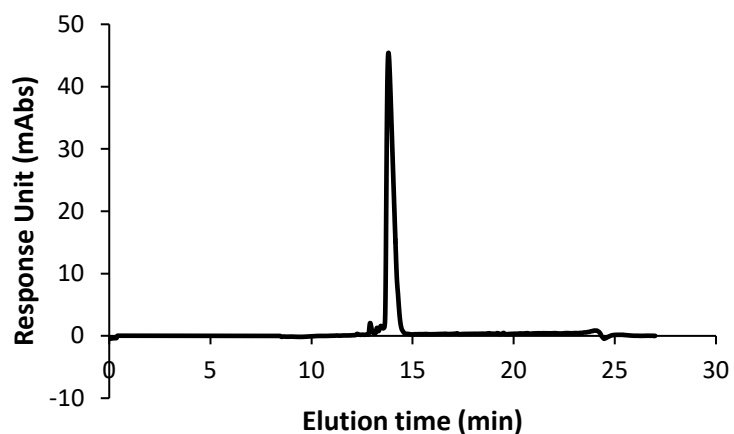
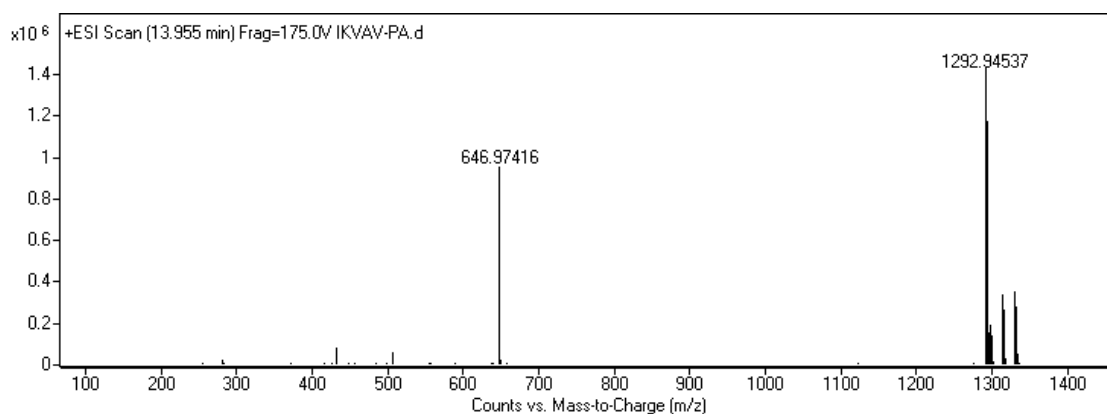


Fig. S1 (A) Liquid chromatogram and (B, C) mass spectra of K₂PA. (B) [M+H]⁺ (calculated): 782.58 , [M+H]⁺ (observed): 782.59, [M+2H]²⁺/2 (calculated): 391.79, [M+2H]²⁺/2(observed): 391.80. (C) [M+H]⁺ (calculated): 782.58 , [M+H]⁺ (observed): 782.59.

(A)



(B)



(C)

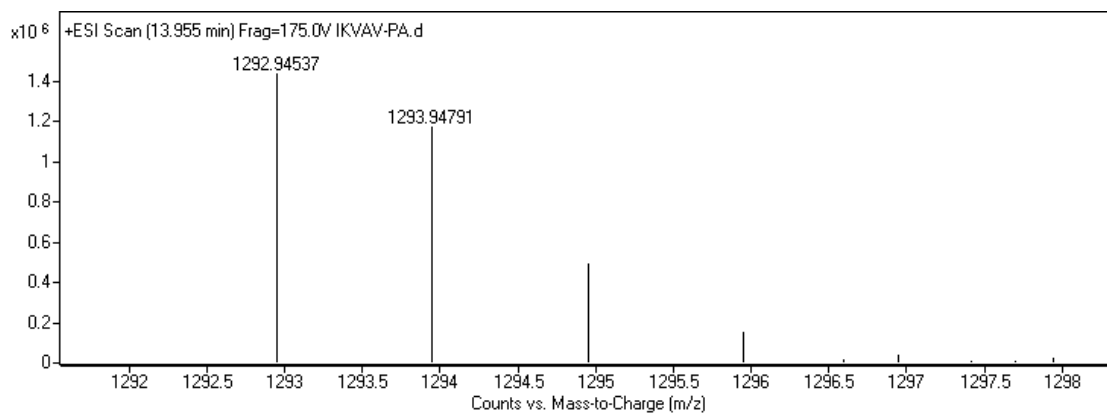


Fig. S2 (A) Liquid chromatogram and (B, C) mass spectra of K_2PA -IKVAV. (B) $[M+H]^+$ (calculated): 1292.93, $[M+H]^+$ (observed): 1292.94, $[M+2H]^{+2}/2$ (calculated): 646.96, $[M+2H]^{+2}/2$ (observed): 646.97. (C) $[M+H]^+$ (calculated): 1292.93, $[M+H]^+$ (observed): 1292.94.

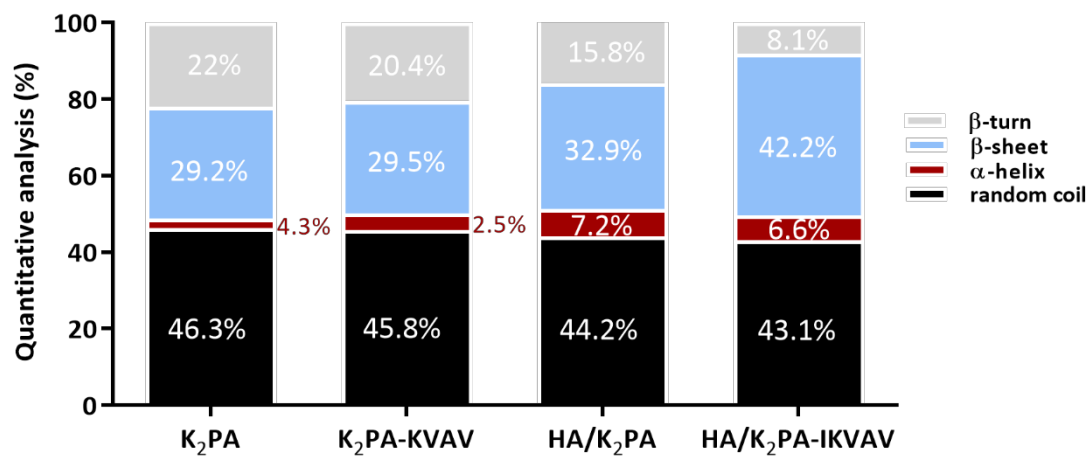


Fig. S3 Quantitative analysis of the CD spectra of individual K₂PA and K₂PA-IKVAV molecules and HA/K₂PA and HA/K₂PA-IKVAV co-assembled supramolecular systems (1:1 v/v).

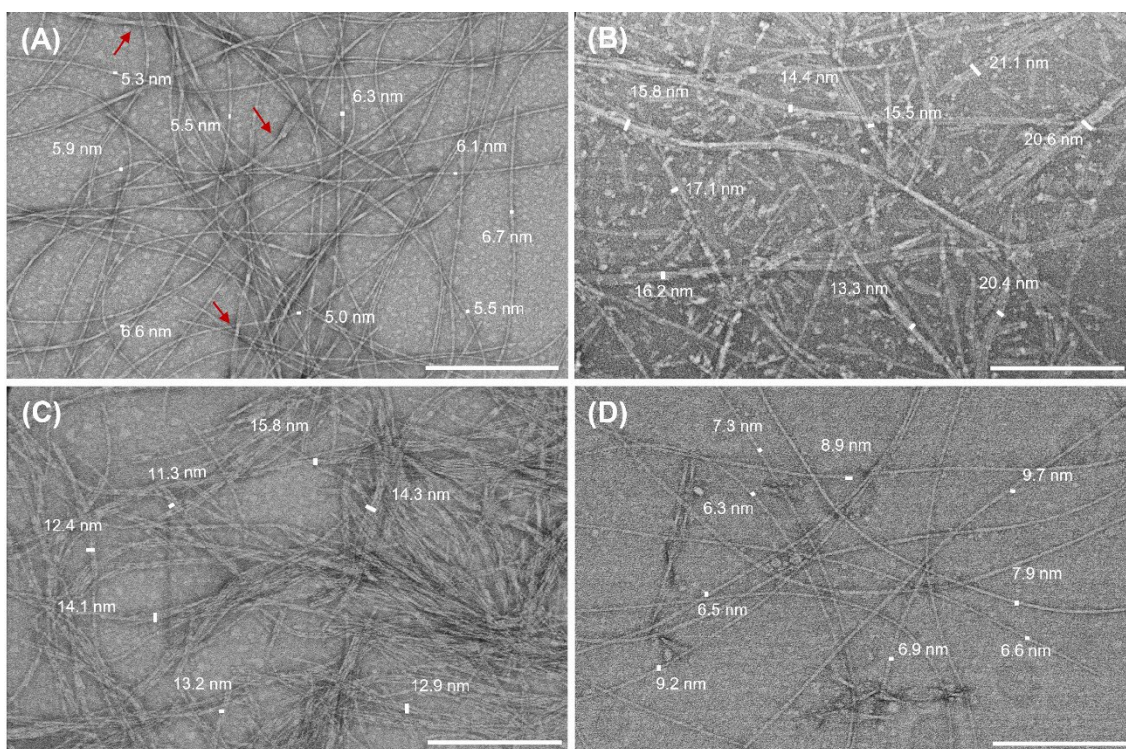


Fig. S4 Representative TEM micrographs of the nanofibrous structure formed by (A) K_2PA -IKVAV, (B) K_2PA , (C) HA/ K_2PA -IKVAV, and (D) HA/ K_2PA systems (1:1 v/v ratio) at 0.2 mg/mL, with nanofiber widths highlighted in white and twisted nanofibers highlighted with red arrows. The magnification factor is x100k. Scale bars: 300 nm.

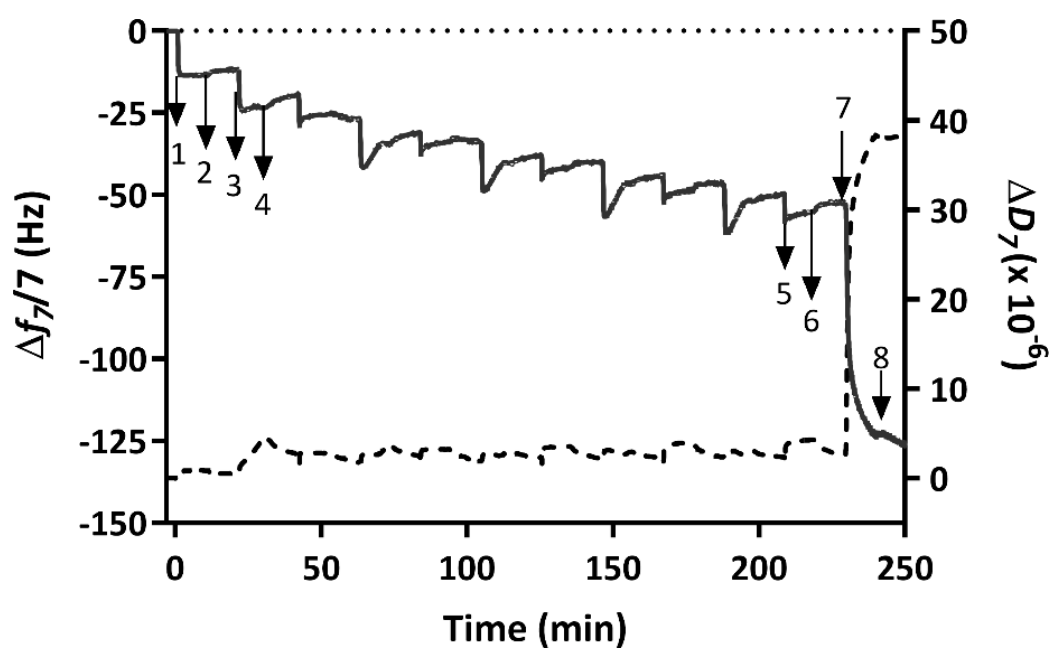


Fig. S5 Build-up of the supramolecular multilayered thin films. Real time QCM-D monitoring of the normalized frequency ($\Delta f_n/n$; solid line) and dissipation (ΔD_n ; dashed line) changes, obtained at the 7th overtone ($n = 7$; 35 MHz), as a function of time for the build-up of (PLL/HA)₅/PLL multilayered nanofilms onto Au-coated quartz crystal sensors and subsequent adsorption of an K₂PA-IKVAV outer layer. Numbers refer to the adsorption of PLL (1, 5), HA (3), K₂PA-IKVAV (7), and rinsing steps (2, 4, 6 and 8).

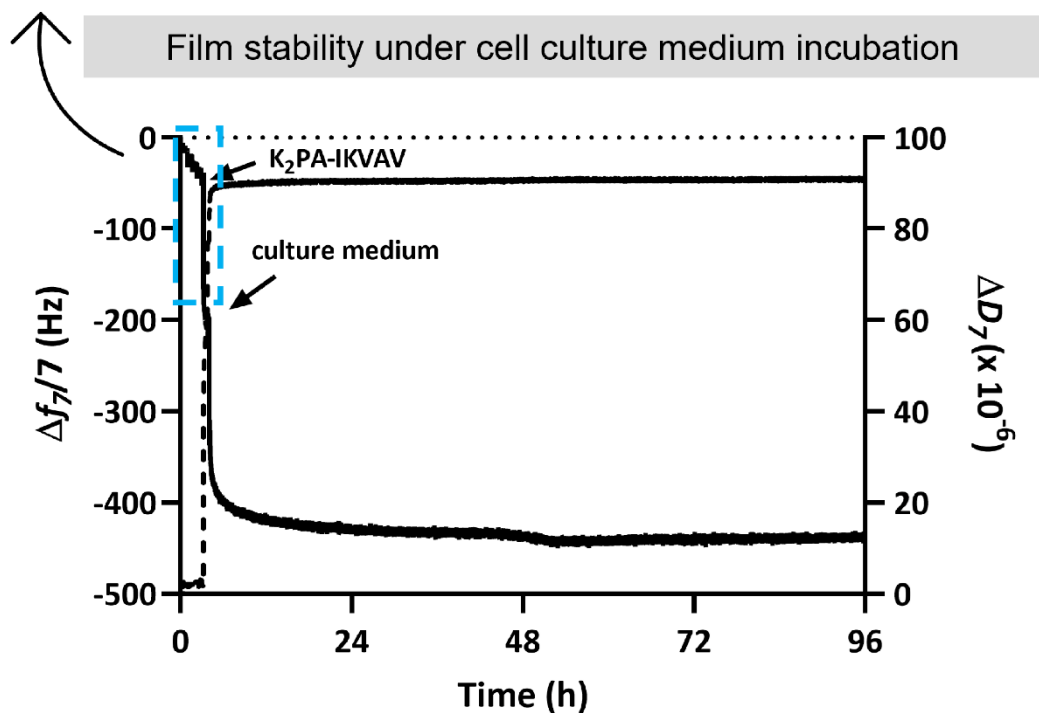
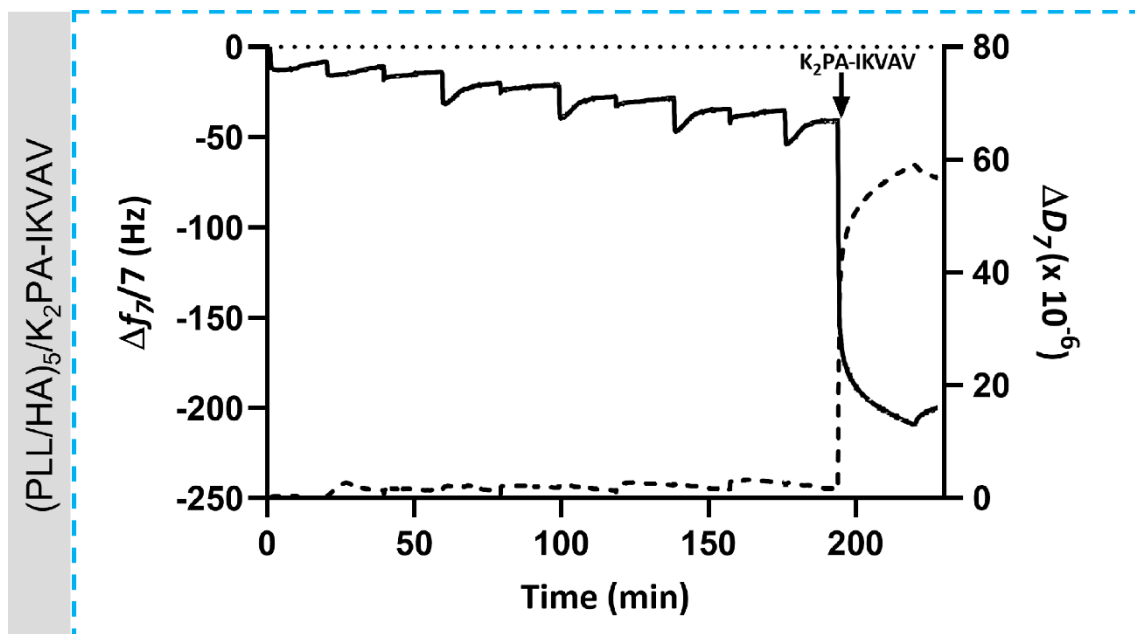


Fig. S6 QCM-D study of the stability of the supramolecular multilayered films. Build-up of the (PLL/HA)₅ multilayered nanofilm onto Au-coated quartz crystal sensors and subsequent adsorption of K₂PA-IKVAV molecules as the outer layer (top image), and further exposure of the film to cell culture medium for 4 days (bottom image). The $\Delta f_n/n$ (solid line) and ΔD_n (dashed line) changes as a function of time were obtained at the 7th overtone ($n = 7$; 35 MHz).

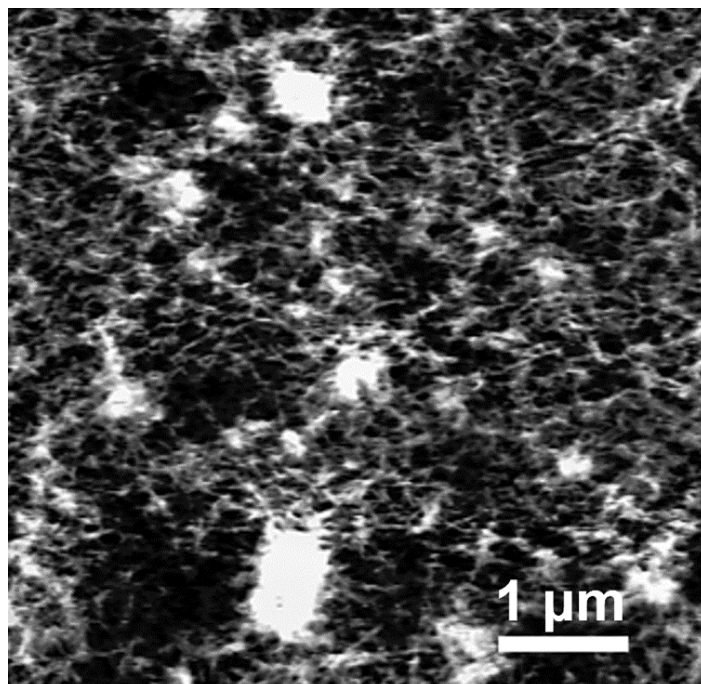


Fig. S7 Representative AFM 2D topographic image for the (PLL/HA)₅/K₂PA-IKVAV functionalized Au substrate suggesting the deposition of nanofibrillar-like structures above the biopolymeric film surface. The original image was converted to grey, corrected for uneven illumination and background subtracted using ImageJ software. Scale bar: 1 μm.

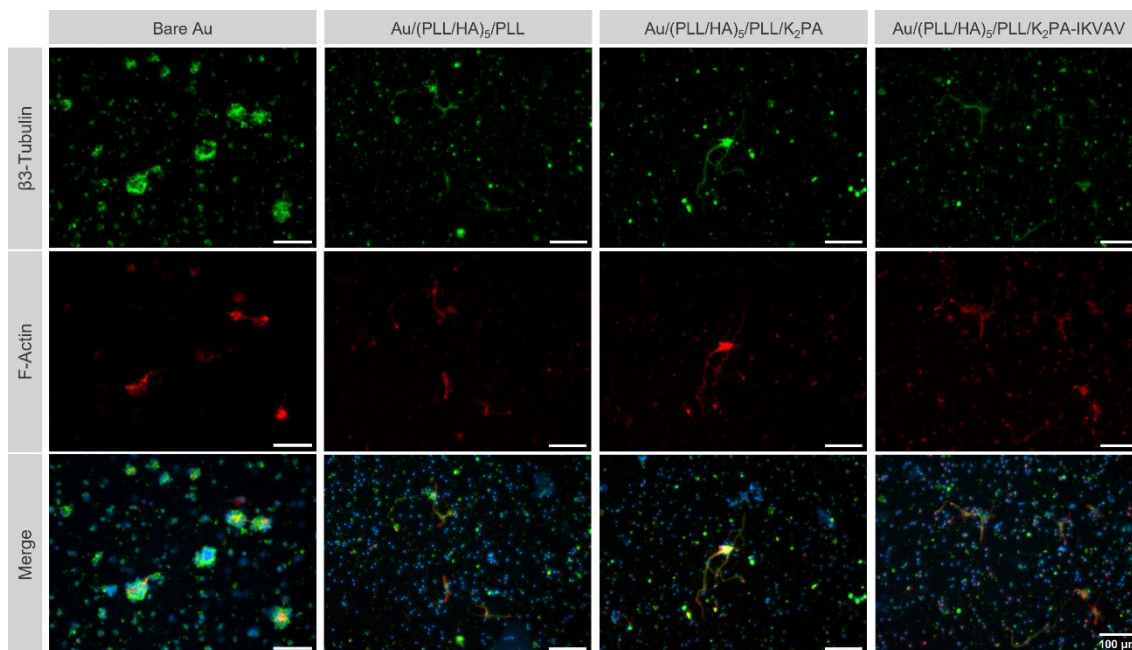


Fig. S8 Morphology of primary neuronal cortical cells at 4 days of *in vitro* culture on uncoated and LbL coated Au surfaces. Representative fluorescence microscopy images of primary neuronal cortical cells at 4 days of culture on the bare Au substrate, and (PLL/HA)₅/PLL, (PLL/HA)₅/PLL/K₂PA and (PLL/HA)₅/PLL/K₂PA-IKVAV functionalized Au substrate, after immunostaining with β-III tubulin (green), f-actin filaments (red), and nuclei counterstaining with Hoechst (blue). Scale bars: 100 μm.