Electronic Supporting Information for:

Self-assembled hybrid hydrogels based on an amphipathic low molecular weight peptide derivative and a water-soluble poly(para-phenylene vinylene)

Jenifer Rubio-Magnieto,[a*] Marta Tena-Solsona,‡[a,b] Beatriu Escuder,[b*] and Mathieu Surin[a]

[a] *Laboratory for Chemistry of Novel Materials, Center for Innovation in Materials and Polymers, University of Mons - UMONS, 20 Place du Parc, B-7000 Mons, Belgium. Tel: +32 65 373868; E-mail: Jenifer.rubiomagnieto@umons.ac.be*
[b] *Departament de Química Inorgànica i Orgànica, Universitat Jaume I, Avenida Sos Baynat s/n, 12071 Castelló, Spain. E-mail: escuder@uji.es.*
‡ Present address: Fakultät für Chemie, Technische Universität München, Lichtenbergstraße 4, 85748 Garching, Germany.

Table of contents:

Experimental Section

Synthesis and Characterization

Reagents and solvents were purchased from commercial suppliers (Aldrich, VWR) and were used without further purification.

Synthesis of compound ZFKFK has been previously reported in "M. Tena-Solsona et al., *Chem. Eur. J.*, **2014**, 20, 1023" by using a step by step synthetic procedure in solution. ^{1}H and ^{13}C NMR are in agreement with the literature spectra.

The poly[5-methoxy-2-(3-sulfopropoxy)-1,4-phenylenevinylene] potassium salt (**PPV**) was purchased from Sigma Aldrich, with a concentration of 0.25 wt. % in H_2O and was used as received.

Preparation of the tetrapeptide: Gelation experimental procedure. Preparation of the tetrapeptide-PPV mixtures

The exact amount of ZFKFK compound was dissolved in TRIS·HCl buffer 100 mM (pH 7.4) at three different concentrations (0.6 mM, 6 mM and 15 mM), which are below and above the minimum gelling concentration of the peptide derivative, *i.e.* around 13 mM (determined by using the test tube inversion method). To obtain mixed hydrogels of ZFKFK:PPV, solutions of ZFKFK were gently heated until complete dissolution was achieved. A small and exact volume of aqueous solution of PPV (0.22 mM) was added over warm solutions of peptide and the mixtures were left to equilibrate during 20 minutes at room temperature in order to form the gels.

UV-Vis absorption and Circular Dichroism spectroscopy

The UV-Vis absorption and circular dichroism (CD) measurements were recorded using a Jasco J-810 Spectrometer. The measurements were carried out using 1 mm suprasil quartz cells from Hellma Analytics. The spectra were recorded between 190 and 600 nm, with a bandwidth of 1 nm, 500 nm/min and 5 repetitions. Buffered water solvent reference spectra were used as baselines and were automatically subtracted from the CD spectra of the samples.

Variable temperature experiments (VT-CD) were performed using a PTC-423S Temperature Controller from Jasco running on the Jasco J-810 Spectrometer. The temperatures were varied from 20 ºC to 65 ºC heating/cooling cycles at a rate of 1 ºC/min with a stabilization time of 10 minutes and 5 minutes, for the heating and cooling cycles, respectively.

Fluorescence spectroscopy

Emission spectra were recorded using a Perkin-Elmer LS55 spectrophotometer and a Chirascan™ Plus instrument which allows fluorescence measurements. Fluorescence measurements were carried out at 25 ºC by using a 10 mm quartz cells (1 mL) from Lightpath Optical. The excitation wavelength was set at 451 nm, which is the maximum absorption wavelength of the polymer. The spectra were recorded between 475 and 700 nm, with a bandwidth of 10 nm and time per point of 1 s.

Atomic Force Microscopy

For AFM measurements, solutions were deposited on freshly-cleaved mica substrates .AFM images were acquired in air at room temperature, using an ICON AFM system (Bruker Corporation, Santa Barbara, CA, USA) operating in the peak force tapping mode. AFM images were recorded over 1−10 μm^2 with 512 × 512 pixels at a scanning speed of 0.7 Hz using a SNL cantilever (0.12 N/m tip). The images were analyzed and processed using the Nanoscope image analysis program.

Transmission Electron Microscopy (TEM)

Samples (gels or solutions) were applied directly onto Formvar carbon film on 200 mesh copper grids. Excess gel was carefully removed by capillarity. After that one drop of distillated water was added in order to remove salts and excess solvent was removed again in the same manner. The grids were immediately stained with one drop of phosphotungstic acid 1 % for 5 min. Excess stain was removed by capillary action. TEM images were recorded in a Transmission Electron Microscope JEOL 2100.

Methylene Blue Release (MB)

Gels of ZFKFK at 15 mM were prepared in TRIS·HCl buffer 100 mM by using the heating-cooling methodology previously described. In this case, immediately after the complete solution, samples were transferred to a UV-cuvette and MB (25 μM) and the corresponding amount of PPV (0 or 0.012 mM) were added while the self-assembly process takes place. After 24 h of stabilization at room temperature 1 mL of water was added and the release of dye was monitored in situ following the absorbance of the supernatant at $\lambda = 624$ nm for the MB. Blank in water containing MB at the same concentrations was prepared in order to normalize the percentage of release.

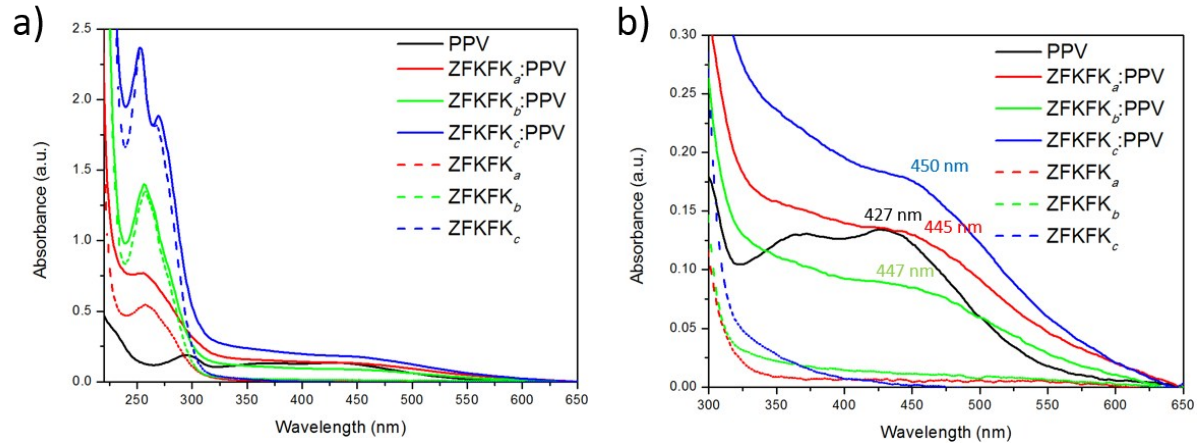

Figure S1. a) UV-Vis and b) Zoom of the UV-Vis spectra of 0.003 mM PPV into a solution of ZFKFK (in which *a* is 0.6 mM, *b* is 6 mM and *c* is 15 mM).

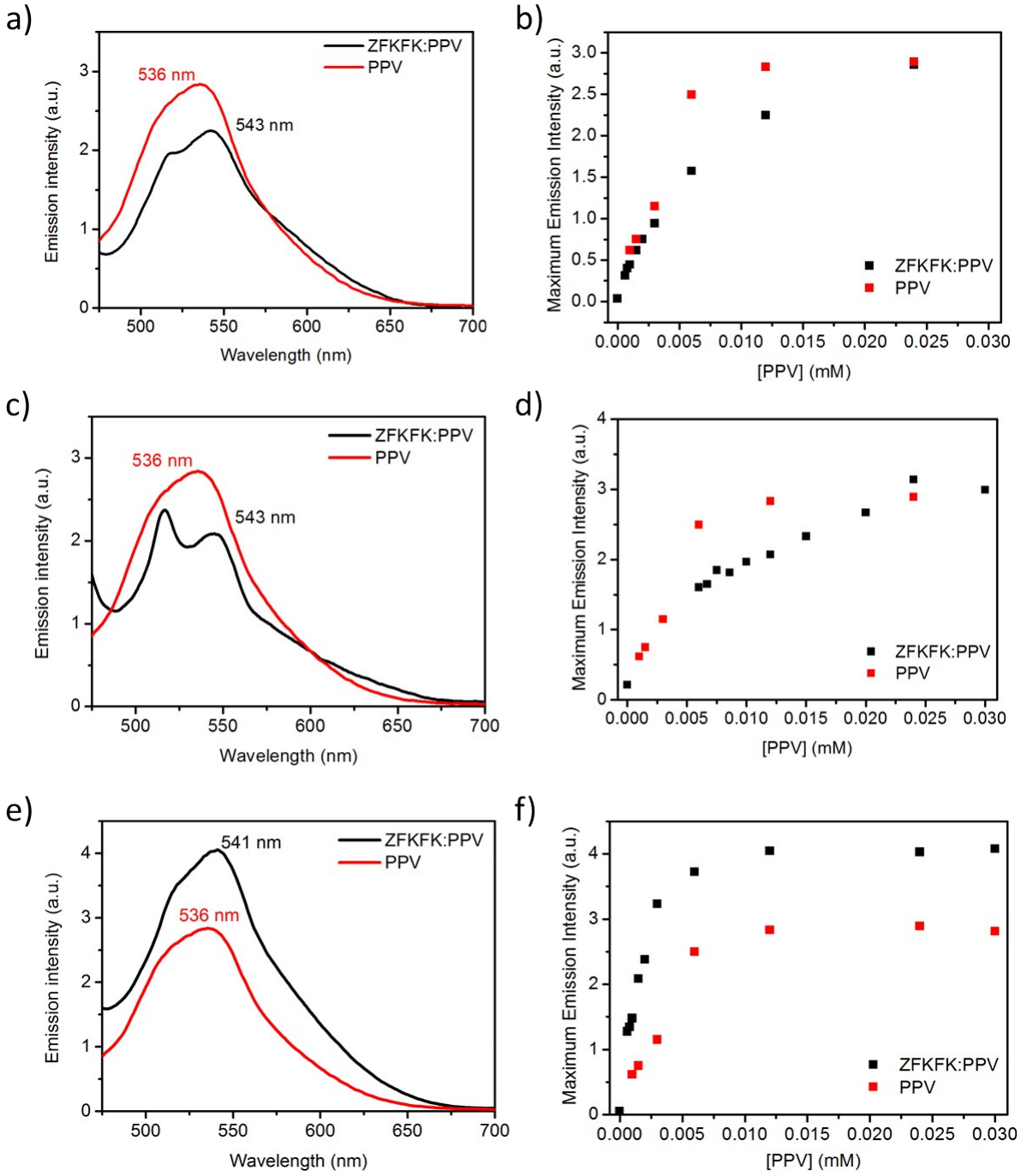

Figure S2. a) Emission spectra (λ_{exc} = 451 nm) of 0.012 mM PPV into a solution of 0.6 mM of ZFKFK (molar ratio 1:50 in PPV:ZFKFK); b) Intensity of emission spectra at 540 nm (λ_{exc}= 451 nm) of PPV into a solution of 0.6 mM peptide at different molar ratio concentration. c) Emission spectra (at λ_{exc} = 451 nm) of 0.012 mM PPV into a solution of compound 6 mM of ZFKFK (molar ratio 1:500 in PPV:ZFKFK); d) Intensity of emission spectra at 540 nm (λ_{exc} = 451 nm) of PPV into a solution of peptide (black squares) at different molar ratio and PPV at different concentration (red squares). e) Emission spectra (at λ_{exc} = 451 nm) of 0.012 mM PPV into a solution of compound 15 mM of ZFKFK (molar ratio 1:1250 in PPV:ZFKFK); f) Intensity of emission spectra at 540 nm (λ_{exc} = 451 nm) of PPV into a solution of peptide (black squares) at different molar ratio and PPV at different concentration (red squares).

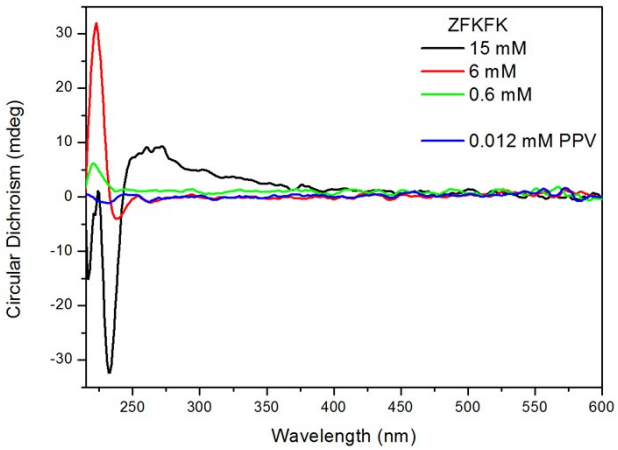

Figure S3. Circular Dichroism (CD) spectra of ZFKFK:PPV mixture at different concentrations of tetrapeptide and at 20 ºC in Tris buffer. Concentration of PPV is fixed at 0.012 mM for each spectrum.

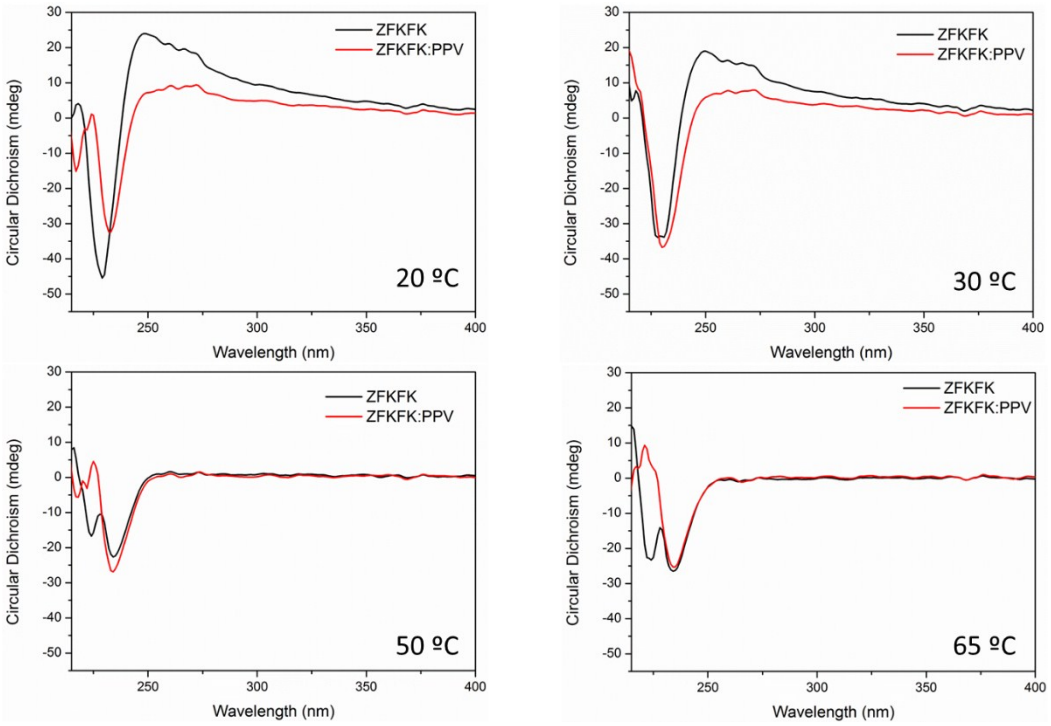

Figure S4. Variable-temperature Circular Dichroism (VT-CD) spectra (Heating Cycle) of a) pure ZFKFK and b) ZFKFK:PPV mixture at 15 mM of tetrapeptide and at different temperatures in Tris buffer. Concentration of PPV in the mixture is fixed at 0.012 mM for each spectrum.

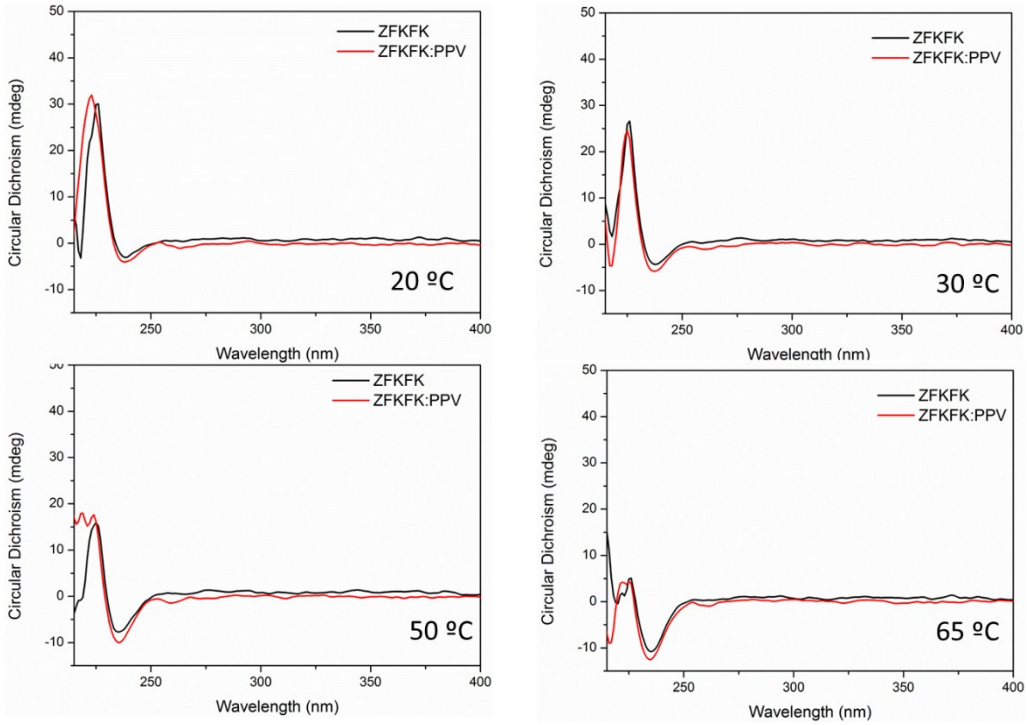

Figure S5. Variable-temperature Circular Dichroism (VT-CD) spectra (Heating Cycle) of a) pure ZFKFK and b) ZFKFK:PPV mixture at 6 mM of tetrapeptide and at different temperatures in Tris buffer. Concentration of PPV in the mixture is fixed at 0.012 mM for each spectrum.

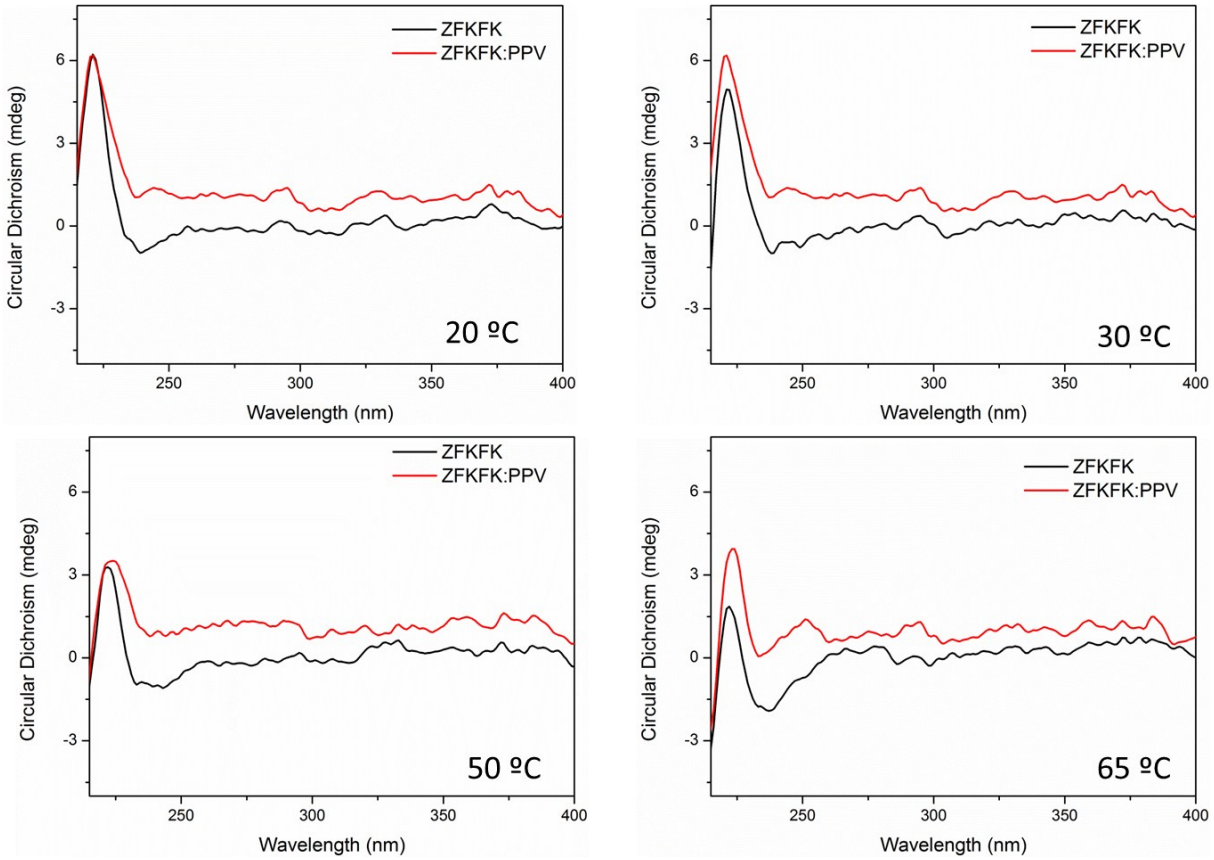

Figure S6. Variable-temperature Circular Dichroism (VT-CD) spectra (Heating Cycle) of a) pure ZFKFK and b) ZFKFK:PPV mixture at 0.6 mM of tetrapeptide and at different temperatures in Tris buffer. Concentration of PPV in the mixture is fixed at 0.012 mM for each spectrum.

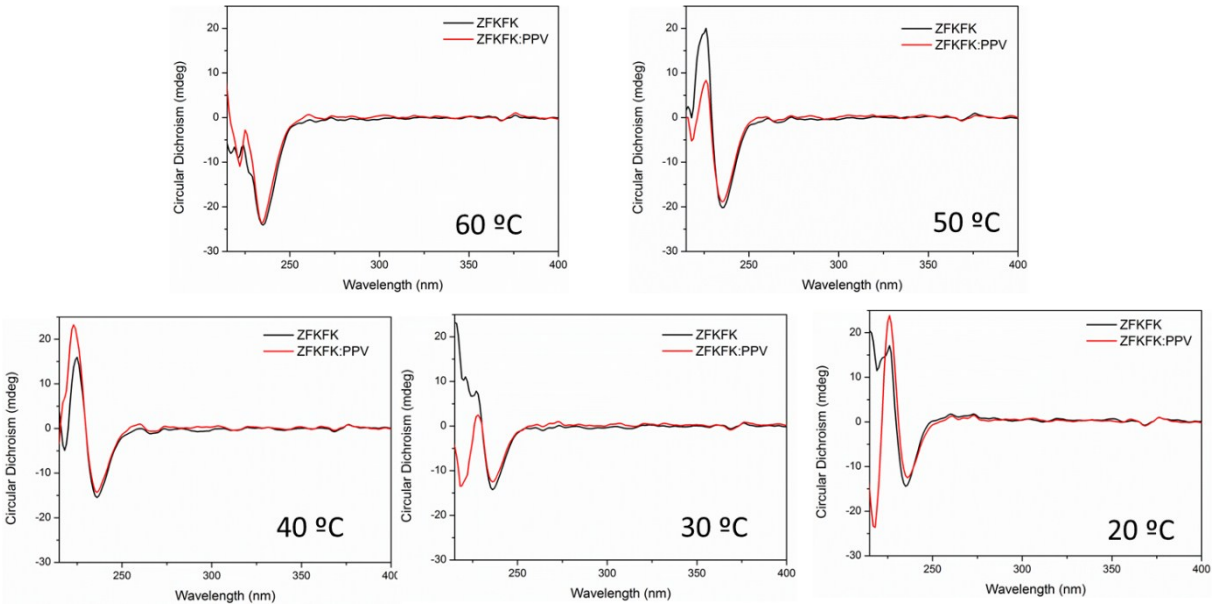

Figure S7. Variable-temperature Circular Dichroism (VT-CD) spectra (Cooling Cycle) of a) pure ZFKFK and b) ZFKFK:PPV mixture at 15 mM of tetrapeptide and at different temperatures in Tris buffer. Concentration of PPV in the mixture is fixed at 0.012 mM for each spectrum.

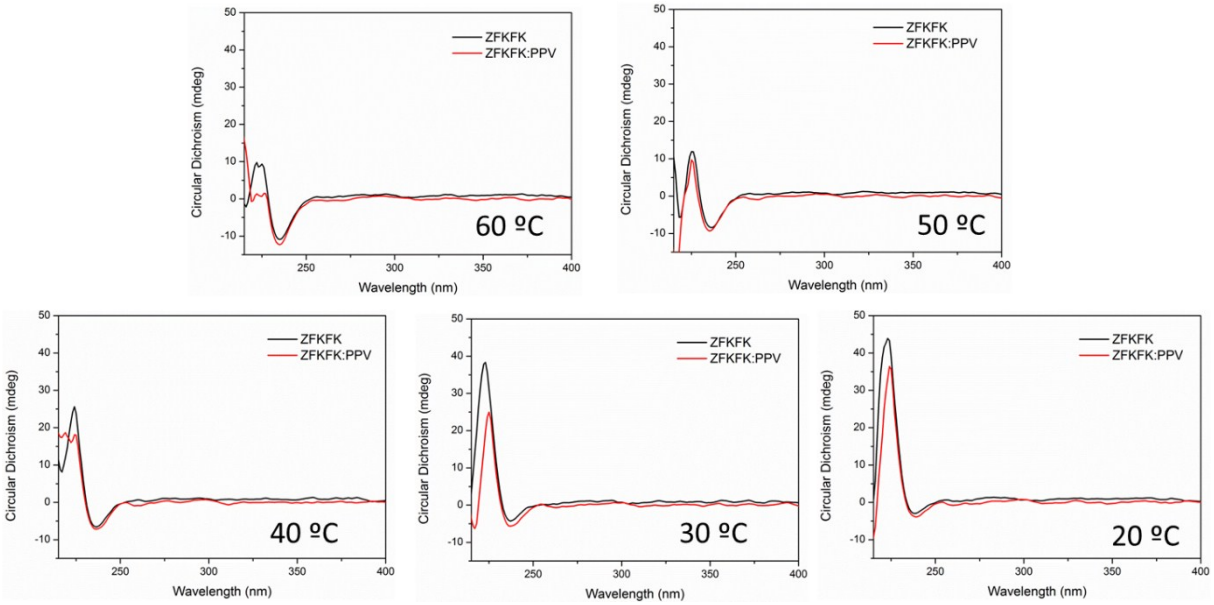

Figure S8. Variable-temperature Circular Dichroism (VT-CD) spectra (Cooling Cycle) of a) pure ZFKFK and b) ZFKFK:PPV mixture at 6 mM of tetrapeptide and at different temperatures in Tris buffer. Concentration of PPV in the mixture is fixed at 0.012 mM for each spectrum.

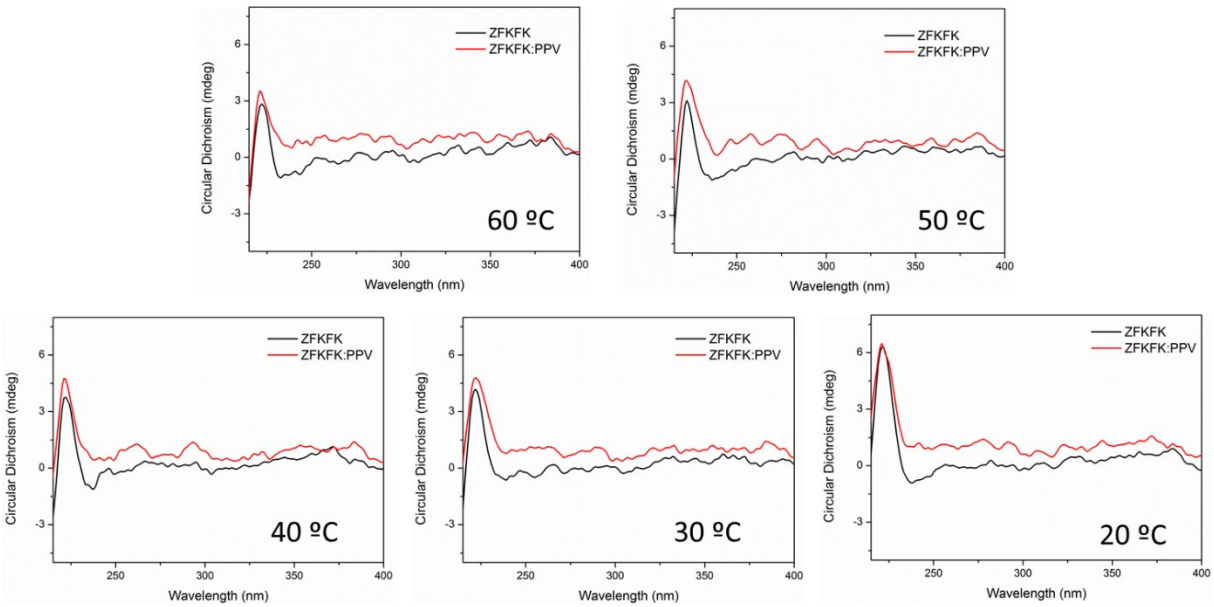

Figure S9. Variable-temperature Circular Dichroism (VT-CD) spectra (Cooling Cycle) of a) pure ZFKFK and b) ZFKFK:PPV mixture at 0.6 mM of tetrapeptide and at different temperatures in Tris buffer. Concentration of PPV in the mixture is fixed at 0.012 mM for each spectrum.

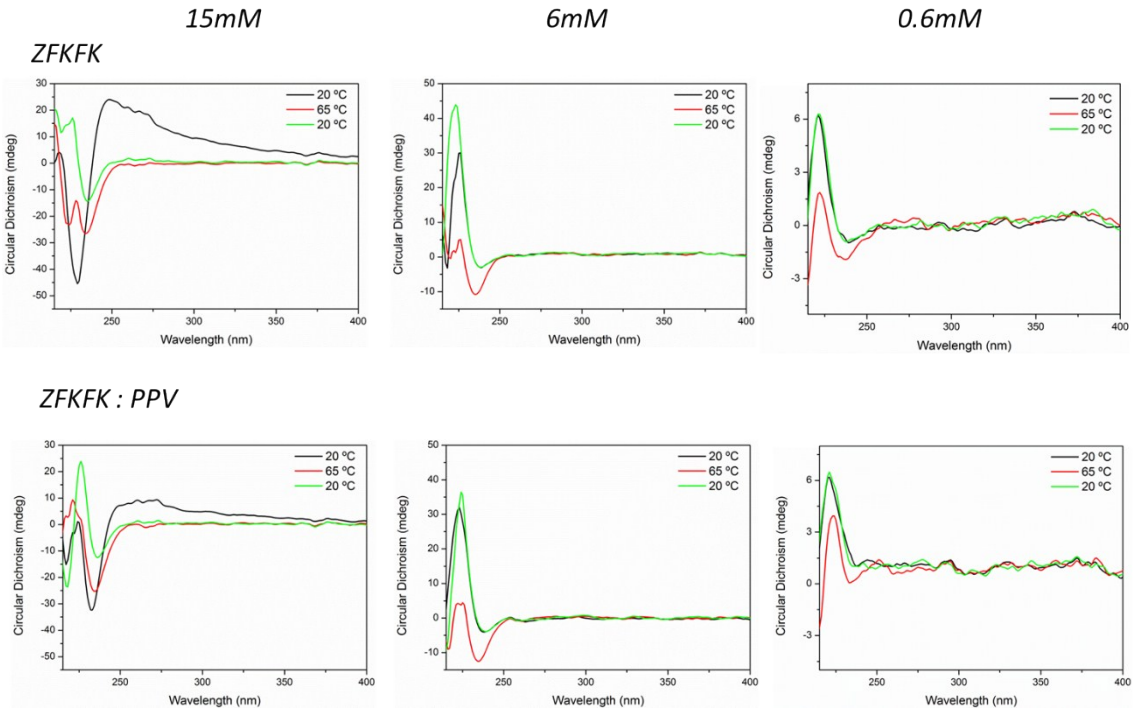

Figure S10. Circular Dichroism (CD) spectra of pure ZFKFK (top) and ZFKFK:PPV mixture (bottom) at different concentrations of tetrapeptide (15 mM, 6 mM and 0.6 mM) and at 20 ºC (before heating cycle), 65 ºC (after heating cycle) and 20 ºC (after cooling cycle) in Tris buffer. Concentration of PPV in the mixture is fixed at 0.012 mM for each spectrum.

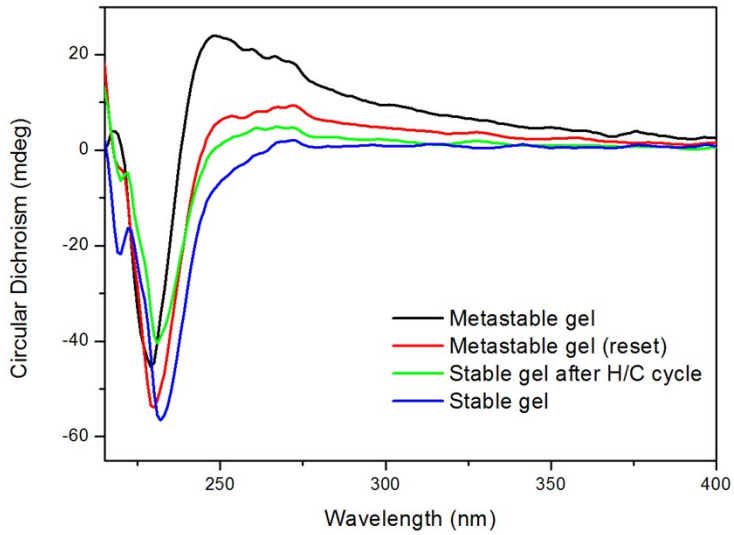

Figure S11. Circular Dichroism (CD) spectra of pure ZFKFK at 15 mM and at 20 ºC in Tris buffer. The CD curves show different gel structures.

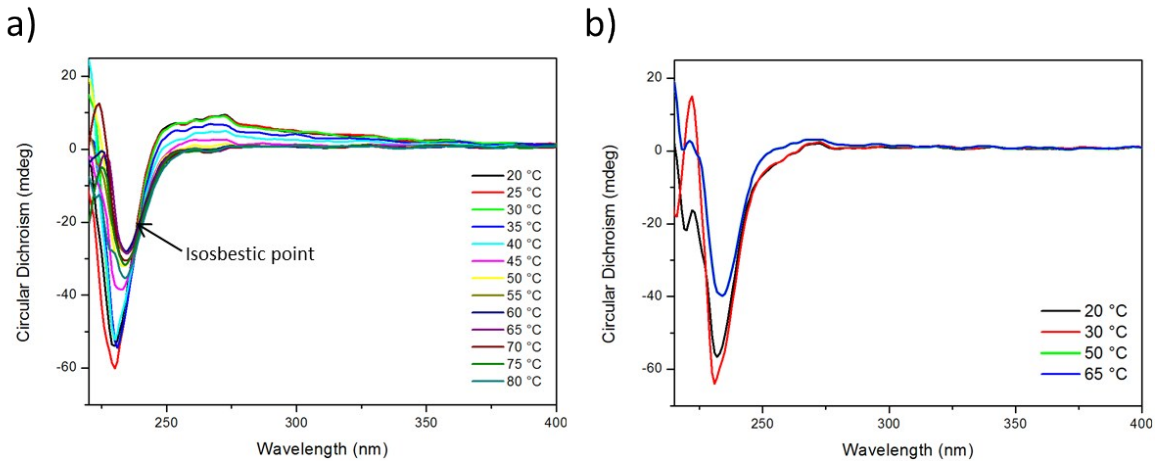

Figure S12. Variable-temperature Circular Dichroism (VT-CD, heating cycle) spectra of a) metastable and b) stable ZFKFK at 15 mM and at 20 ºC in Tris buffer.

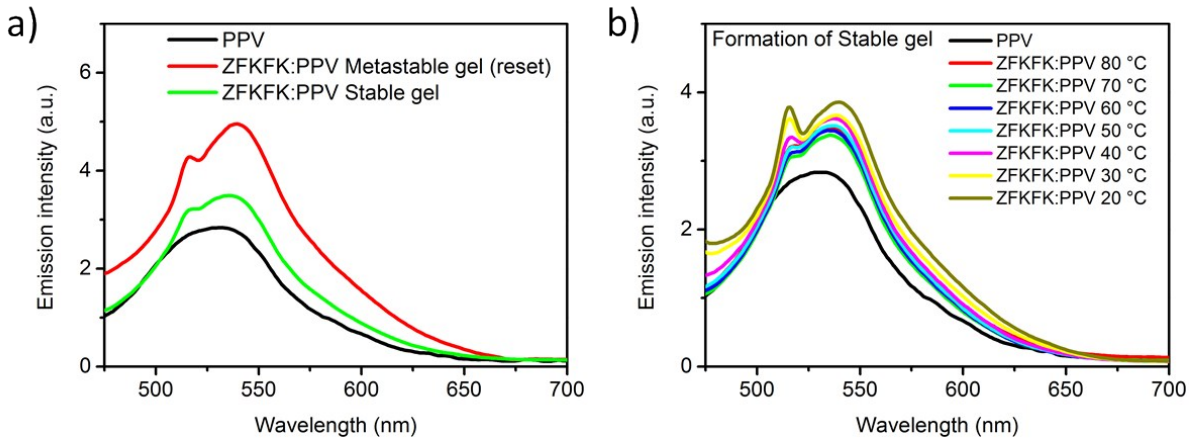

Figure S13. a) Emission spectra (λ_{exc} = 451 nm) of 0.012 mM PPV into a solution of compound 15 mM of ZFKFK (molar ratio 1:1250 in PPV:ZFKFK) in the metastable state and stable state at 20 ºC in Tris buffer; b) Formation of the Stable gel by a cooling ramp.

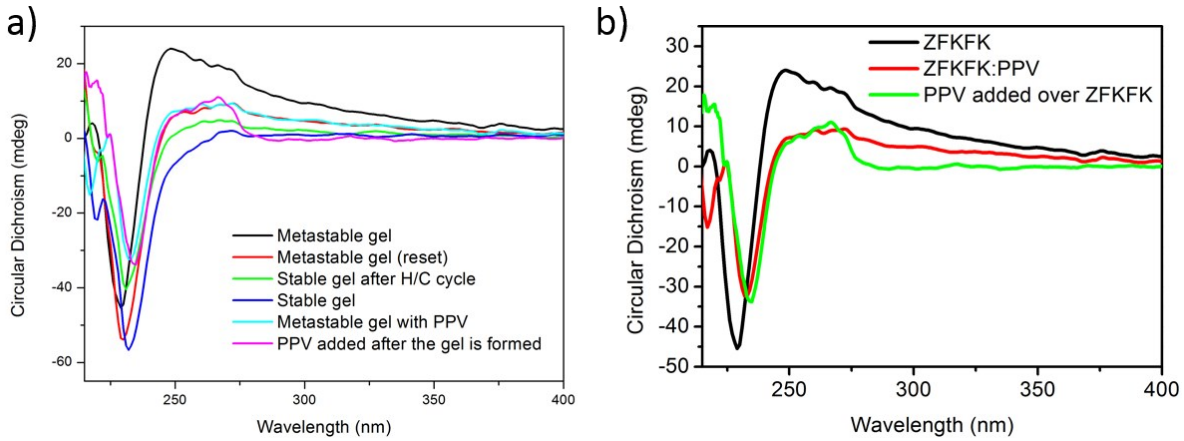

Figure S14. a) Circular Dichroism (CD) spectra of pure ZFKFK at 15 mM showing different gels structures at 20 ºC in Tris buffer; and CD spectra of the ZFKFK:PPV when the polymer solution is mixed with an already formed metastable gel (pink line). b) Circular Dichroism spectra of pure ZFKFK (black line), ZFKFK:PPV (red line) and ZFKFK:PPV when the polymer solution is mixed with an already formed metastable gel (green line). The concentration of PPV and ZFKFK are fixed at 0.012 mM and 15 mM, respectively.

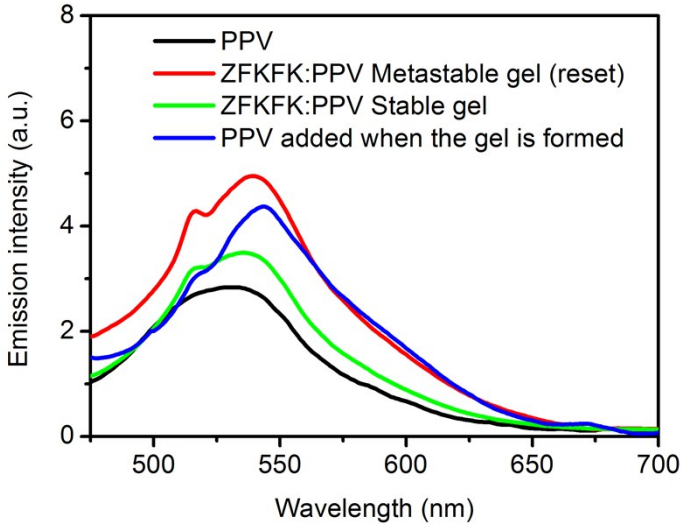

Figure S15. Emission spectra (λ_{exc} = 451 nm) of 0.012 mM PPV into a solution of compound 15 mM of ZFKFK (molar ratio 1:1250 in PPV:ZFKFK) in the metastable state (red line), stable state (green line) and when the PPV solution is mixed with an already formed metastable gel (blue line) at 20 ºC in Tris buffer.

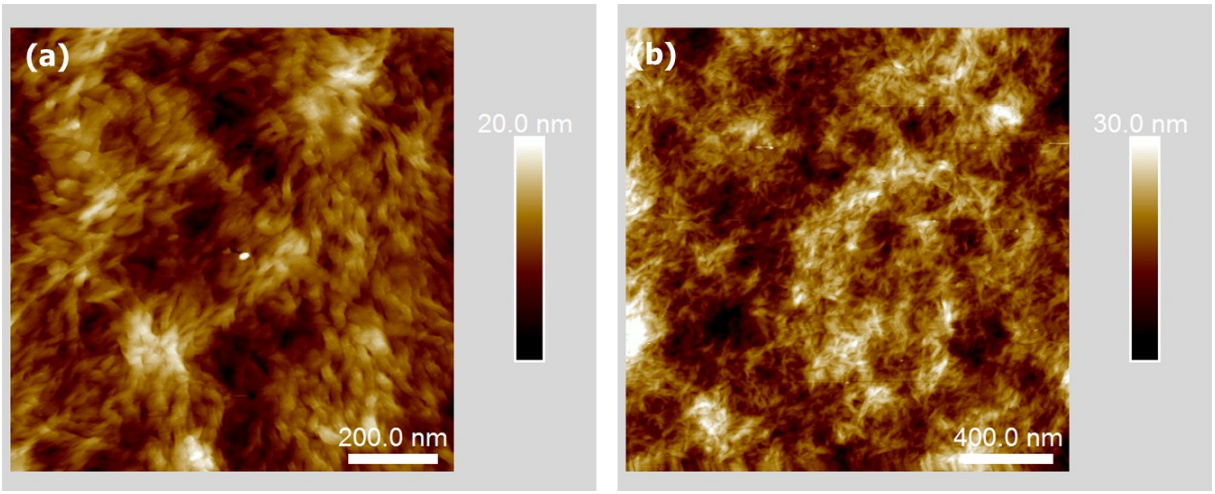

Figure S16. AFM height images of a thin deposit of 1 mM ZFKFK on mica.

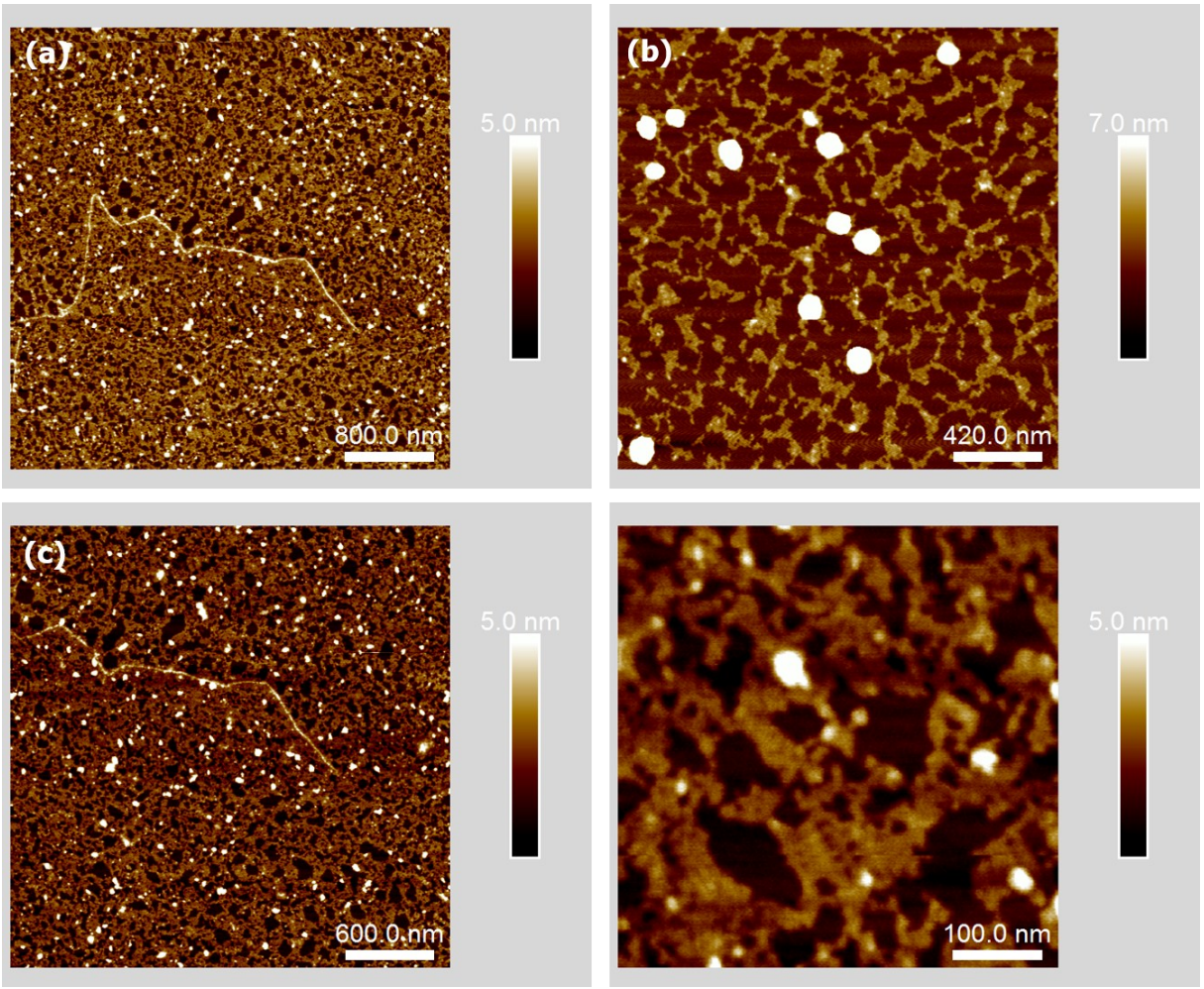

Figure S17. AFM height images of a thin deposit of 0.01 mM PPV on mica.

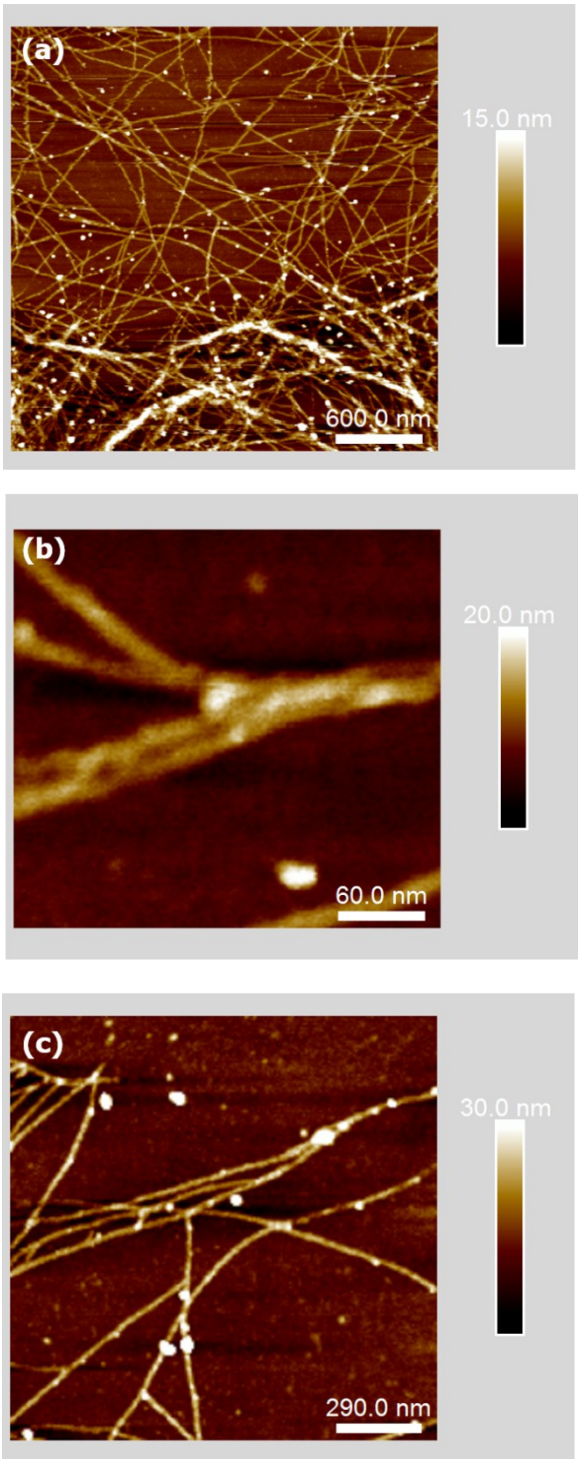

Figure S18. AFM height images of a thin deposit of a mixture of ZFKFK:PPV at 1:0.01.

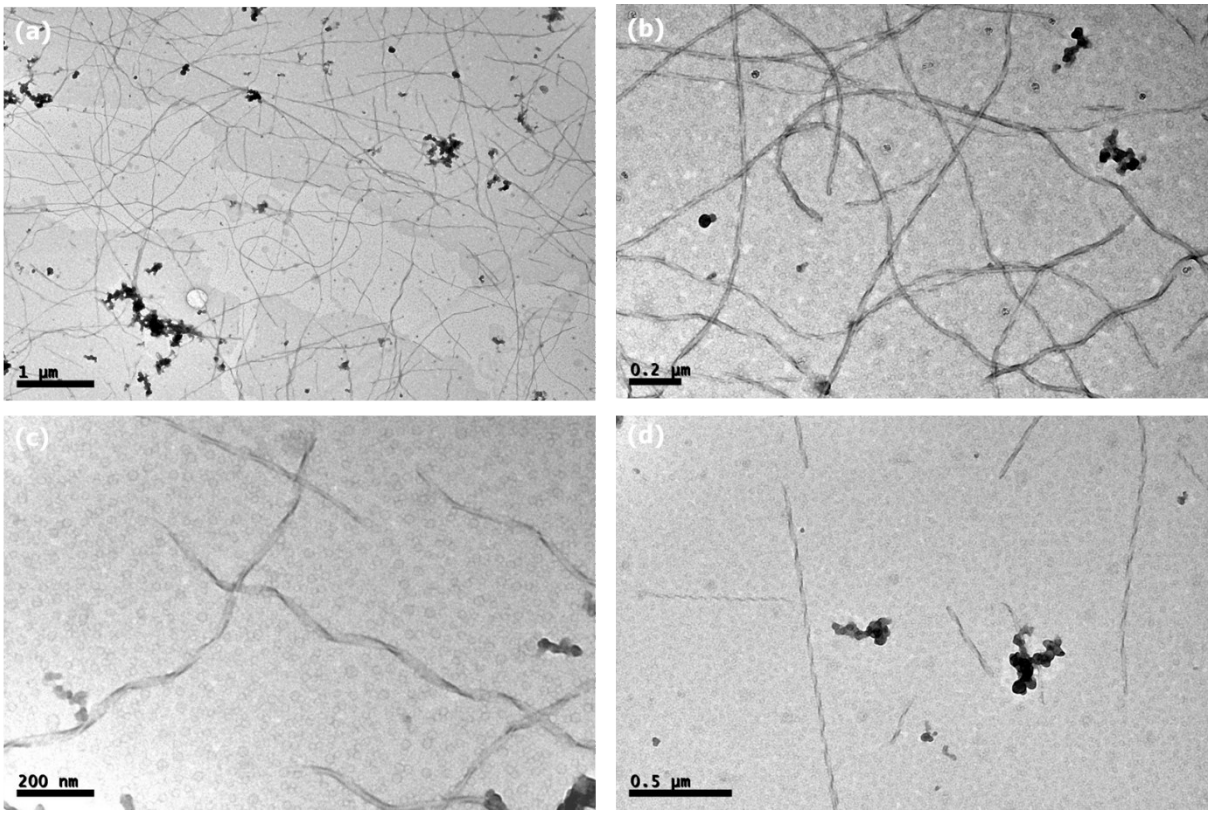

Figure S19. TEM micrographs of xerogel of ZFKFK prepared from hydrogel at 15 mM.

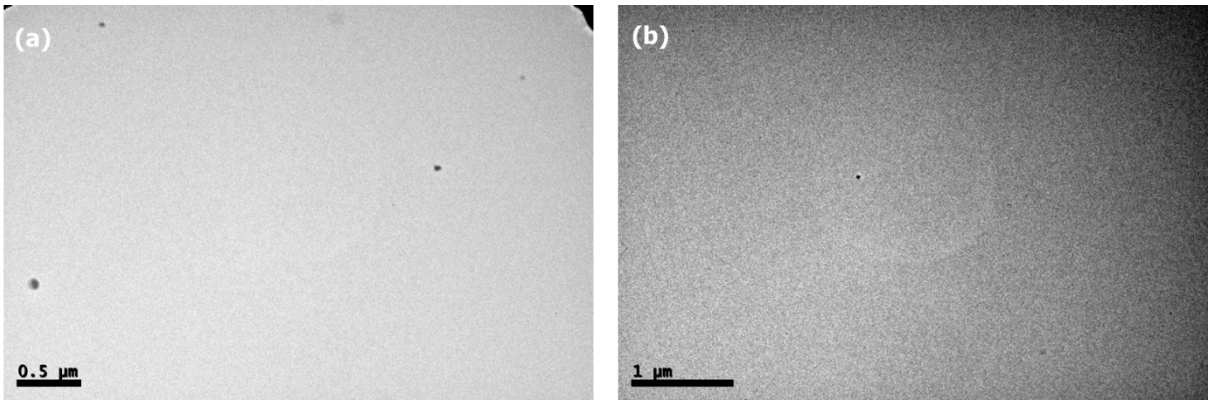

Figure S20. TEM micrographs of PPV prepared from a PPV solution at 0.012 mM.

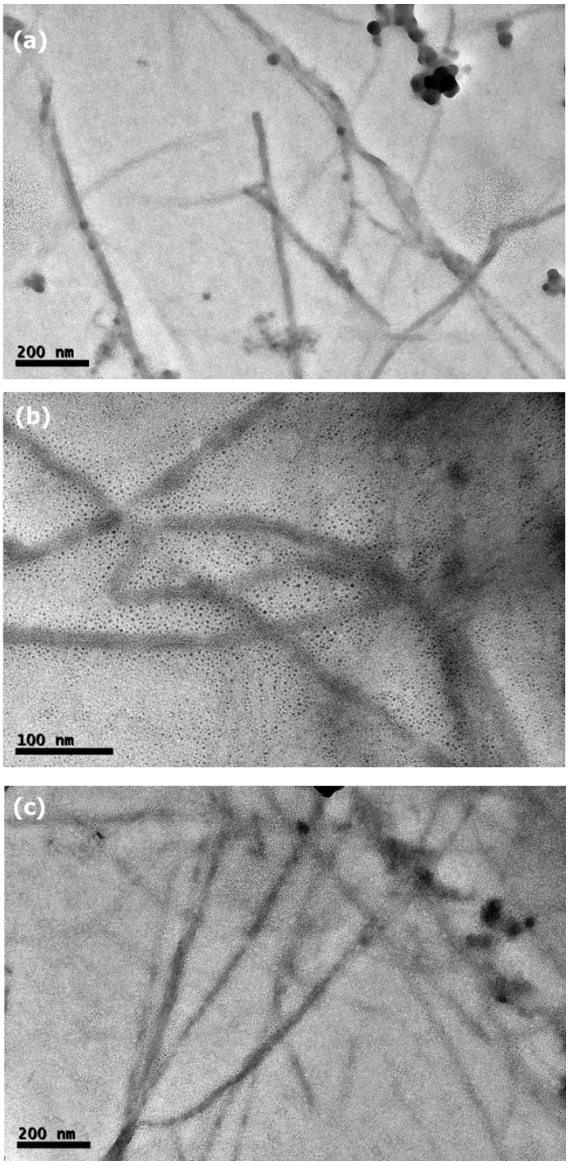

Figure S21. TEM micrographs of a mixture of ZFKFK and PPV prepared from hydrogel at 15 mM and 0.012 mM PPV.

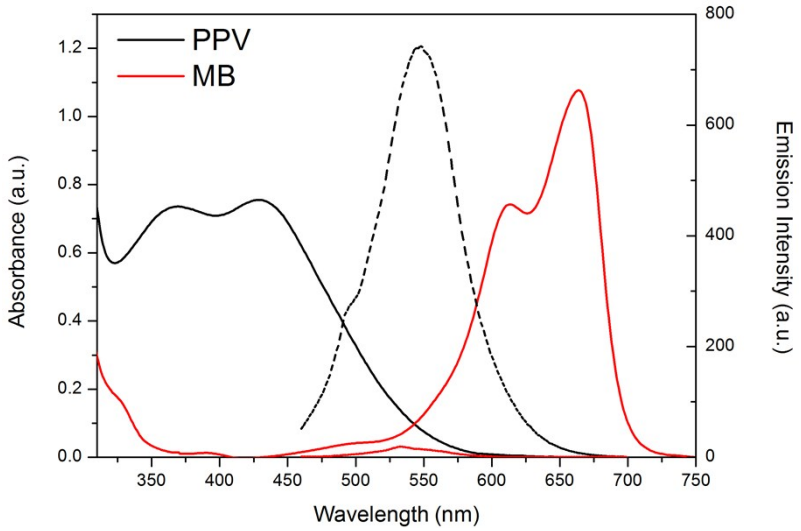

Figure S22. Absorption (solid lines) and emission (dash lines) spectra of pure PPV (black lines) and pure MB (red lines). [MB] = 25 μM and [PPV] = 0.012 mM.

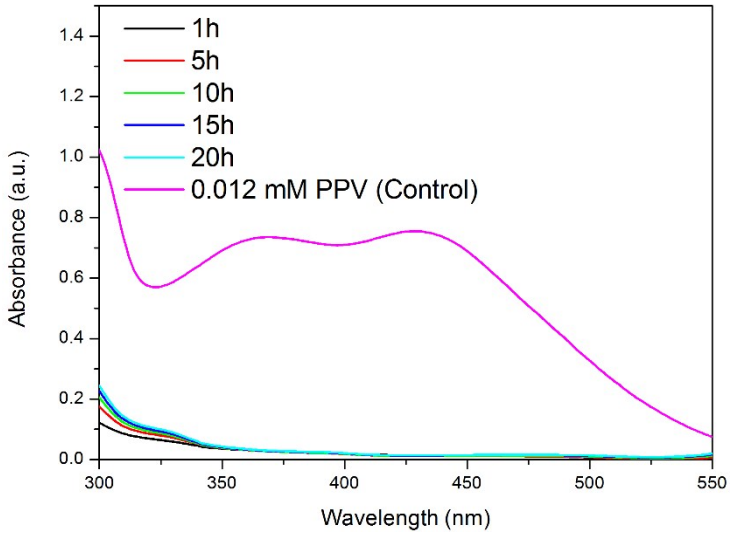

Figure S23. UV-Vis spectra of ZFKFK:PPV:MB mixture after addition of 1 mL of Tris buffer at different times. [ZFKFK] = 15 mM, [MB] = 25 μM and [PPV] = 0.012 mM. The magenta line corresponds to the spectrum of PPV at 0.012 mM (the concentration used in the release experiment), and it can be used as a control in order to prove that there is no evidence of PPV in the supernatant solution.

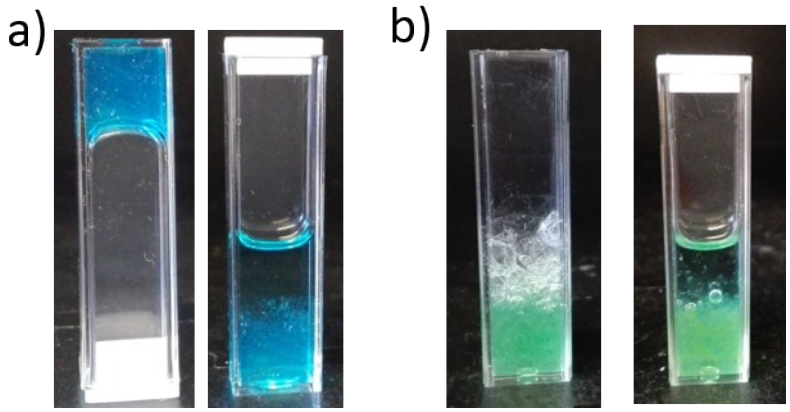

Figure S24. Macroscopic aspect of a) ZFKFK and b) ZFKFK:PPV hydrogels containing MB, before and 20 h after the addition of Tris buffer. [ZFKFK] = 15 mM, [MB] = 25 μM and [PPV] = 0.012 mM.