Supplementary Information

Two-step Kinetic Model as Self-assembling Mechanism for Diphenylalanine Micro/Nanotubes Formation

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1. Images of FF-film and tubes formation

To ensure the reproducibility of the experiments, a micro-syringe (62RNR, Hamilton) was built up on a xyz-stage to control the position of the water drop relative to laser spot position at the sample (Fig. S1). We checked whether the position of syringe needle was set in the same position for every measurement by using the Raman microscope camera with a 10X air objective (N.A. 0.25, MPlan N, Olympus). The distance between the top-right corner of the needle and laser spot position (green circle on Fig. S1 a), was approximately 415 μ m. To investigate the effect of water diffusion, we followed the video image obtained with the 40X objective and verified how the added water behaved during the formation process of FF-MNTs. Therefore, a water drop was placed onto the film at t = 0 s and as consequence the whole area that was being observed was covered by water (Fig. S1–b). After 4s it is possible to observe a change in the appearance of the film and emergence of tubes in subsequent times (Fig. S1– c-h). We notice that the formed structures remained unchanged and mechanically stable which indicates absence of diffusion or other transport phenomena influence on the formation of the FF-MNTs or in the formation of intermediate species.



Figure S1 - Images from Raman microscope camera. a) Syringe needle position relative to the laser spot at the film which is represented by the green circle at the center of the image, b-h) Sequential pictures taken every 4s from the moment the water was added (t = 0 s) to moments after the emergence of FF-MNTs.

2. Polarized Rama spectra

Raman spectra of the FF-MNTs were obtained by applying parallel and perpendicular laser polarization relative to the tube axis. Spectra were normalized by 1583 cm⁻¹ and 1603 cm⁻¹ peak, since they are insensitive to the polarization.



Figure S2: Raman spectra of the FF-MNTs corresponding to parallel and perpendicular laser polarization configuration relative to the tube axis.

3. Time dependent frequency shift and FWHM of additional vibrational modes

The other two modes that present a similar two-step frequency behavior of the 1000 cm⁻¹ mode (Fig. S3-a) and time-dependence of frequency and bandwidth correspondent to the emergent sub-peak at 1037 cm⁻¹, after t=8 s (Fig. S3-b).



Figure S3: a) Time-dependence of 1583 and 1603 cm⁻¹ and b) 1037 cm⁻¹ sub-peak mode frequencies. The left blue scale represents the FWHM of the mode

4. Time dependent frequency shift of 1000 cm⁻¹ mode and relative concentration of species

Frequency shift analysis (Fig. S4a-c) and the estimated relative concentration of initial, intermediate and final species (Fig. S4d-f) during the formation process were also performed for another three measurement sets. All parameter values used for the simulation such as frequency and FWHM of 1000 cm⁻¹ mode at t = 0, 5 and 30 s, corresponding to the 3 observed species, decay rate and critical time are reunited in Table S1, including for the measurement set 1, shown in Fig 3.



Figure S4: a-c) Simulation of frequency shift of different measurements set during the formation process of the FF-MNT, d-f) relative concentration of first (1), intermediate (2) and final species (3).

Table S1 – Values of the parameters used to perform the simulation of frequency shift of 1000 cm⁻¹ mode and population growth and decay of species.

	Measurement set			
	1 (Fig. 3)	2 (Fig S4a, d)	3 (Fig S4b,e)	4 (Fig S4c, f)
$v_{t=0s}$ (cm ⁻¹)	1002.2	1002.0	1002.1	1002.2
$\overline{v}_{t} = 5s(\text{cm}^{-1})$	1001.3	1001.1	1001.1	1001.3
$\overline{v}_{t=30s}$ (cm ⁻¹)	1000.4	1000.0	1000.3	1000.4
$FWHM_{t=0s}$ (cm ⁻¹)	2.9	2.9	2.9	2.9
$FWHM_{t=5s}$ (cm ⁻¹)	3.0	3.0	3.0	3.0
$FWHM_{t=30s}$ (cm ⁻¹)	2.2	2.7	2.6	2.4
$k_1(s^{-1})$	1.5	2.2	1.0	0.9
$k_2(s^{-1})$	1.0	0.2	0.4	0.6
$\tau_{c1}(s)$	1.5	1.5	1.0	0.5
$\tau_{c2}(s)$	8.5	14.5	10.0	10.0

5. Vibrational modes calculations

The FF monomers crystallize with hydrogen-bonded head-to-tail chains in the form of helices with six dipeptide molecules per turn (Fig. S5) and a channel core with a diameter of 10 Å, that is filled with water molecules (1). Small peptides structures enclosing up to six FFs (1FF, 2FF, ..., 6FF) and FF-H₂O units were cropped from this core-motif to produce the starting structures for vibrational calculation.



Figure S5. Core motif of FF nanotubes in the basal plane. The unit cell is formed by six molecules of FF arranged in a hexagonal pack.

The structures were pre-optimized in a first step using molecular mechanics with the MMFF94s force field (2) implemented in the Avogadro software (3). Density Functional Theory (DFT) (4, 5) was used in order to obtain the equilibrium geometries and harmonic frequencies. The convergence of the calculation was also checked by computing the total energy as a function of unit cell volume variation. Calculations were implemented in the CPMD program (6) using the BLYP functional (7) augmented with dispersion corrections for the proper description of van der Waals interactions (8, 9) For all simulations, the cutoff energy was considered up to 100 Ry. The wave functions were optimized and then the vibrational modes were obtained with Raman responses using the Hessian matrix. The linear response for the values of polarization and polar tensors of each atom in the system was calculated to evaluate the eigenvectors of each vibrational mode. The criteria for vibrational modes assignment were both frequency value proximity for smallest structures to experimental frequency value (within 5%) and searching for identical eigenvectors in the more complex structures. Only eigenvectors for those bands related to the FF-nanotubes growing process (Table 1) will be presented.

- B mode

We assigned this vibration to the complex mode evolving the donor water hydrogen and torsional modes occurring in the $FF-H_2O$ subunit at 741 cm⁻¹. Figure S6 presents the eigenvectors for this mode.



Figure S6. Eigenvectors for the calculated B mode associated to the FF-water interaction. This mode was associated to the 761 cm⁻¹ experimentally observed band.

- C mode

This mode is associated to an inclined C-H bending. Note that the eigenvectors are inclined from the ring plane normal. The eigenvalues presented a broad and consistent decreasing with the dipeptide content corroborating the experimentally observed data.



Figure S7. Eigenvectors for those modes associated to the C mode.

- E mode

This corresponds to a pucker phenyl ring with eigenvectors oscillation with a small projection along the direction perpendicular to the plane. This mode is absent in the 1FF case.



Figure S8. Eigenvectors for those modes associated to the E mode.

6. Raman Spectral Evolution of FF MNTs formation



Figure S9. Time-spectral evolution of a typical Raman measurement of FF-MNTs formation. a) Complete spectra obtained from t = 0 s to t = 20 s (Δ t = 1 s), b-d) Peaks of interest are represented by vertical dotted lines and are shown in 3 selected ranges of frequency (displayed with Δ t = 2 s for clarity). For display purpose, spectra have been normalized at 1000 cm⁻¹ and vertically offset for clarity.

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