Nanostructures that are inaccessible through spontaneous thermodynamic processes may be formed by supramolecular self-assembly under kinetic control. In the past decade, the dynamics of pathway complexity in self-assembly have been elucidated through kinetic models based on aggregate growth by sequential monomer association and dissociation. Immiscible liquid|liquid interfaces are an attractive platform to develop well-ordered self-assembled nanostructures, unattainable in bulk solution, due to the templating interaction of the interface with adsorbed molecules. Here, we report time-resolved in situ UV/vis spectroscopic observations of the self-assembly of zinc(II) meso-tetrakis(4-carboxyphenyl)porphyrin (ZnTPPc) at an immiscible aqueous|organic interface. We show that the kinetically favoured metastable J-type nanostructures form quickly, but then transform into stable thermodynamically favoured H-type nanostructures. Numerical modelling revealed two parallel and competing cooperative pathways leading to the different porphyrin nanostructures. These insights demonstrate that pathway complexity is not unique to self-assembly processes in bulk solution, and equally valid for interfacial self-assembly. Subsequently, the interfacial electrostatic environment was tuned using a kosmotropic anion (citrate) in order to control the influence the pathway selection. At high concentrations, interfacial nanostructure formation was forced completely down the kinetically favoured pathway and only J-type nanostructures were obtained. Furthermore, we found by atomic force microscopy (AFM) and scanning electron microscopy (SEM) that the J- and H-type nanostructures obtained at low and high citric acid concentrations, respectively, are morphologically distinct, which illustrates the pathway-dependent material properties.
Pathway Complexity in Supramolecular Porphyrin Self-Assembly at an Immiscible Liquid|Liquid Interface

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Abstract

Nanostructures that are inaccessible through spontaneous thermodynamic processes may be formed by supramolecular self-assembly under kinetic control. In the past decade, the dynamics of pathway complexity in self-assembly have been elucidated through kinetic models based on aggregate growth by sequential monomer association and dissociation. Immiscible liquid|liquid interfaces are an attractive platform to develop well-ordered self-assembled nanostructures, unattainable in bulk solution, due to the templating interaction of the interface with adsorbed molecules. Here, we report time-resolved in situ UV/vis spectroscopic observations of the self-assembly of zinc(II) meso-tetrakis(4-carboxyphenyl)porphyrin (ZnTPPc) at an immiscible aqueous|organic interface. We show that the kinetically favoured metastable J-type nanostructures form quickly, but then transform into stable thermodynamically favoured H-type nanostructures. Numerical modelling revealed two parallel and competing cooperative pathways leading to the different porphyrin nanostructures. These insights demonstrate that pathway complexity is not unique to self-assembly processes in bulk solution, and equally valid for interfacial self-assembly. Subsequently, the interfacial electrostatic environment was tuned using a kosmotropic anion (citrate) in order to control the influence the pathway selection. At high concentrations, interfacial nanostructure formation was forced completely down the kinetically favoured pathway and only J-type nanostructures were obtained. Furthermore, we found by atomic force microscopy (AFM) and scanning electron microscopy (SEM) that the J- and H-type nanostructures obtained at low and high citric acid concentrations, respectively, are morphologically distinct, which illustrates the pathway-dependent material properties.
Introduction

Self-assembly is a powerful route to access elaborate, functional supramolecular nanostructures from relatively simple molecules.\textsuperscript{1–4} The properties of these nanostructures, and ensuing performance characteristics in device applications, depend on the precise molecular organisation of the individual building-blocks.\textsuperscript{5} Supramolecular polymers are a key subclass of self-assembled nanostructures, defined as one-dimensional arrays of monomeric units that are interconnected by reversible and highly directional secondary interactions such as hydrogen bonds, metal-ligand coordination, \(\pi-\pi\) stacking or combinations thereof.\textsuperscript{6,7}

Over the past decade, kinetic studies probing the time-dependent behaviour of supramolecular polymers composed of, \textit{e.g.}, porphyrin,\textsuperscript{8–12} bis(merocyanine),\textsuperscript{13} oligo(paraphenylenevinylene)\textsuperscript{14,15} or perylene bisimide dyes,\textsuperscript{16–18} have comprehensively demonstrated the existence of competing assembly pathways, \textit{i.e.}, pathway complexity. Control over the interplay between these competing pathways is heavily influenced by the preparation methodologies (concentration, temperature, pH, solvent, ionic strength, external stimuli, \textit{etc.}).\textsuperscript{6,19} Thus, manipulation of the latter can potentially lead to nanostructures formed at the thermodynamic equilibrium of the system, or alternatively metastable or kinetically trapped non-equilibrium nanostructures.\textsuperscript{6,19}

The competing assembly pathways that lead to supramolecular polymers can be described by distinct isodesmic or cooperative (nucleation–elongation) mechanisms.\textsuperscript{19–23} In an isodesmic mechanism, the Gibbs free energy of every monomer addition is equivalent, with all individual steps described by a single equilibrium constant (\(K\)).\textsuperscript{24} A cooperative mechanism is characterized by formation of a thermodynamically unfavourable nucleus (or oligomer), followed by energetically favoured elongations steps, and described by two equilibrium constants for the nucleation (\(K_n\)) and elongation steps (\(K_e\)), respectively.\textsuperscript{21} These mechanisms have been distinguished by concentration- and/or temperature-dependent spectroscopic measurements that probe the molecule to nanostructure transition.\textsuperscript{23}

To date, pathway complexity has been described exclusively for systems that self-assemble in bulk solutions. A powerful alternative approach is molecular self-assembly at “soft” liquid|air or immiscible liquid|liquid interfaces.\textsuperscript{25–28} Such “soft” interfaces are considered defect-free, highly reproducible, and self-healing.\textsuperscript{29} These attributes facilitate macroscale uniformity in molecule–interface interactions, providing a route to self-assembled
films of nanomaterials with continuous domains of macroscale (>cm$^2$) long-range order, exhibiting high structural perfection.$^{30}$

Due to their similarities to natural dyes functioning in photosynthetic systems, supramolecular assemblies of zinc(II) 5,10,15,20-(tetra-4-carboxyphenyl)porphyrin (ZnTPPc) molecules at immiscible liquid|liquid interfaces are of particular interest for solar energy conversion and storage applications.$^{31,32}$ Early work demonstrated that photocurrents obtained at porphyrin nanostructure functionalised liquid|liquid interfaces are remarkably dependent on the light polarization, indicating a well-ordered self-assembled nanostructure due to the templating interaction of the interface.$^{33}$ Recently, our group demonstrated that these interfacial ZnTPPc nanostructures are stabilized by cooperative hydrogen bonding and likely represent metastable or kinetically trapped non-equilibrium nanostructures.$^{34,35}$

Despite such insights, our understanding of the assembly mechanism of nanostructures at immiscible liquid|liquid interfaces remains limited. Due to easily detectable spectral changes arising from exciton coupling of their transition dipole moments, dye molecules are ideal candidates to study the mechanisms and thermodynamics of interfacial self-assembly processes by UV/vis spectroscopy. Here we report time-resolved UV/vis spectroscopic observations of the formation of supramolecular assemblies of ZnTPPc at an immiscible liquid|liquid interface as a function of the aqueous pH, porphyrin concentration and electrolyte concentration. Due to the interface’s buried nature, we developed a custom UV/vis setup that operates in total internal reflection mode (TIR-UV/vis absorption) to monitor in situ the evolution with time of the Soret band of a adsorbed interfacial ZnTPPc species. Multiple ZnTPPc nanostructures formed on the interface simultaneously leads to overlapping of their spectrophotometric signals. Thus, the spectral data were analysed by a Multivariate Curve Resolution with Alternating Least Squares (MCR-ALS) decomposition methodology. Quantitative insight into the kinetic experiments was obtained from kinetic model calculations (isodesmic and cooperative, respectively), which revealed two parallel and competing pathways leading to the different ZnTPPc nanostructures. Finally, the citric acid concentration in the aqueous phase was increased to change the chemical environment of the self-assembly process and influence the pathway selection.

**Results and Discussion**

*Spectroscopically monitoring the pH- and concentration-dependency of ZnTPPc interfacial self-assembly.* As discussed in our previous work,$^{35}$ ZnTPPc self-assembles at the
interface between water and an immiscible organic solvent, such as α,α,α-trifluorotluene (TFT), to form highly ordered nanostructures. The self-assembly process is selective to the interface, only taking place when the aqueous pH is within +/- 0.2 units of the pKₐ of the porphyrin’s carboxyl groups (pH 5.8). The electronic transitions of the porphyrin’s Soret band, observed between 410 and 470 nm, are sensitive to its molecular environment and, thus, aggregation state. Therefore, by monitoring the Soret band absorbance in situ at the interface with time (up to 1000 s) by TIR-UV/vis absorption (see experimental setup in Figure S1), we probed the influence of the preparation methodology in terms of pH, bulk aqueous ZnTPPc concentration ([ZnTPPc]_{aq.}) and aqueous electrolyte concentration on the interfacial self-assembly kinetics of ZnTPPc.

The absorbance spectra at pH 5.8 with 8 μM ZnTPPc added to the bulk aqueous phase evolved with time, strongly indicating the formation of multiple interfacial nanostructures (Figure 1A). These spectra can be divided into three sequential steps, each clearly identifiable on the TIR-UV/vis spectra heat-map in Figure 1B. First, a growing band (denoted as B1) with a λ_{max.} at 430 nm was observed (Figure 1C(i)). Given the presence of this band at other pH conditions (discussed vide infra), and the λ_{max.} of ZnTPPc molecules in solution (422 nm at pH 5.8), we attributed B1 to individual ZnTPPc molecules adsorbed at the aqueous|organic interface. These adsorbed molecules can serve as a seed layer for further nanostructure growth.

Second, another growing band (B2) with a λ_{max.} at 442 nm was observed (Figure 1C(ii)). Being red-shifted from B1, this band was associated with the formation of an initial J-type interfacial nanostructure. Finally, a third growing band (B3) with a λ_{max.} at 418 nm appeared (Figure 1C(iii)) and was attributed to the formation of a H-type interfacial nanostructure. These final spectra were quite broad, indicating signal overlapping from multiple interfacial nanostructures. Furthermore, the formation of B3 implied the presence of an isosbestic point at 433 nm and, thus, that partial H-J structural interconversion did not require an intermediate species.
Figure 1. Time dependent TIR-UV/vis spectra of ZnTPPc interfacial self-assembly at an immiscible aqueous|organic interface. (A) The bulk aqueous ZnTPPc concentration ([ZnTPPc]_{aq.}) was 8 μM, the aqueous electrolyte employed was 10 mM citric acid, and the pH was adjusted to 5.8. The organic phase was neat α,α,α-trifluorotoluene (TFT). TIR-UV/vis spectra were taken every 0.5 s for 500 seconds (every 10th spectra is shown for clarity). The red spectrum is that of bulk aqueous ZnTPPc at pH 5.8. The raw spectra were treated in R for smoothing and correcting the drift of the signal (Figure S2). (B) Heat-map of the absorbance between 400 and 470 nm with time, clearly showing the trends in the shift of the λ_max as the dominant ZnTPPc species at the interface changes with time. (C) Schematic representation of the self-assembling behaviour of ZnTPPc at the aqueous|organic interface. The three-stages of self-assembly were identified as: (i) adsorption of monomeric ZnTPPc at the aqueous|organic interface to form a “seed layer” (designated Soret 1, or B1, with a λ_max of 422 nm), (ii) rapid formation of metastable J-type nanostructures (B2, λ_max of 442 nm), and (iii) partial interconversion of the J-type to a H-type nanostructure (B3, λ_max of 418 nm). The associated TIR-UV/vis spectra from (A) are shown below each schematic and arrows indicate the general shift in the λ_max as the dominant spectral features (B1, B2 or B3) evolve with time. An animated version is displayed in the Supporting Information (SI).
Of the parameters evaluated, the self-assembly process was most sensitive to the aqueous pH, in agreement with our previous findings. A range of pH values were investigated between pH 5.0 and 6.8 with 8 μM ZnTPPc added to the bulk aqueous phase (Figure S3). For control experiments in the absence of ZnTPPc, no UV/vis signal was detected in the region of interest. Upon addition of ZnTPPc at pH values marginally (≥0.3 pH units) more acidic or alkali than the pKₐ, a single band with a λₘₐₓ at 430 nm was observed (Figure S3). These bands at 430 nm were distinct from those associated with the bulk aqueous ZnTPPc molecules at each pH value, shown as red spectra in Figure S3, and instead attributed to ZnTPPc monomers adsorbed at the aqueous|organic interface.

To study the effect of porphyrin concentration on the self-assembly kinetics, TIR-UV/Vis spectra were analysed by varying the bulk aqueous ZnTPPc concentration ([ZnTPPc]ₐq.) between 1 and 10 μM at optimal pH 5.8 conditions (Figure S4). Using the isotherm of this biphasic system at pH 5.8, these [ZnTPPc]ₐq. values led to interfacial ZnTPPc concentrations (I[ZnTPPc]) between 0.4 and 4.8 nmol·cm⁻², respectively. At I[ZnTPPc]< 2.6 nmol·cm⁻², only the Soret band of adsorbed ZnTPPc was detected with no change in band intensity after 600 s (Figure S4A-B). Meanwhile, at I[ZnTPPc]> 5 nmol·cm⁻², the volume of the aliquot injected into the system destabilised the baseline, and thus inhibited the acquisition of UV/Vis spectra under TIR conditions. Therefore, to ensure statistically robust TIR-UV/Vis spectra acquisition, the I[ZnTPPc] range was limited between 0.4 and 5 nmol·cm⁻² (in effect a [ZnTPPc]ₐq. range between 5 and 10 μM). Within this selected concentration range, interfacial self-assembly proceeded through the three-stage mechanism discussed vide supra.

Kinetic modelling of interfacial ZnTPPc self-assembly by Multivariate Curve Resolution–Alternating Least Squares (MCR-ALS) analysis. Due to a severe overlapping of the spectrophotometric signals (from B1, B2 and B3 discussed above), a principal component analysis (PCA) was first applied to the TIR-UV/vis spectra obtained at pH 5.8 for I[ZnTPPc] values of 2.6, 4.0 and 4.8 nmol·cm⁻², respectively. The scree plot and representative PCA results for 4.0 nmol·cm⁻² are shown in Figure S5. The analysis revealed two significant interfacial ZnTPPc species, identified as H- and J-type nanostructures, with λₘₐₓ. of 418 and 442 nm, respectively. The pure spectra extracted by PCA of each species for a I[ZnTPPc] of 4.0 nmol·cm⁻² are shown in Figure 2A. Using these spectra as a starting point, an MCR-ALS analysis was run to resolve the kinetic profiles of each species. The resulting concentration profiles show that the interfacial J-type nanostructures rapidly formed, reaching a maximum
concentration after 50 s (Figure 2B). The H-type nanostructures formed slower and presented a clear lag-time, suggesting their formation through a nucleated growth mechanism.\textsuperscript{23} In addition, the growth of H-type nanostructures was accompanied by a decrease in the concentration of J-type until their concentrations equilibrated after 250 s (Figure 2B). The corresponding pure spectra extracted by PCA and subsequent MCR-ALS kinetic analysis for $I_{[ZnTPPc]}$ values of 2.6 and 4.8 nmol·cm\textsuperscript{-2} are shown in Figure S6. The quality control parameters of the MCR-ALS modelling are detailed in Table S1.

Figure 2. Multivariate Curve Resolution–Alternating Least Squares (MCR-ALS) analysis of the kinetics of interfacial ZnTPPc self-assembly. (A) MCR-ALS resolved the pure spectra of the H- and J-type nanostructures for an interfacial ZnTPPc concentration ($I_{[ZnTPPc]}$) value of 4 nmol·cm\textsuperscript{-2} at pH 5.8, and (B) the corresponding kinetic profiles for the H- and J-type nanostructures, respectively. (C, D) Comparison of the kinetic profiles resolved by MCR-ALS for $I_{[ZnTPPc]}$ values of 2.6 (solid line), 4.0 (dashed line) and 4.8 nmol·cm\textsuperscript{-2} (dotted line), respectively, for (C) the J-type nanostructure and (D) the H-type nanostructure. The quality control parameters of the MCR-ALS modelling are detailed Table S1.

Comparisons of the influence of $[ZnTPPc]_{aq.}$ on the behaviour of the kinetic profiles for the J- and H-type nanostructures, respectively, are shown in Figure 2C-D. The J-type nanostructure presented a small lag-time for formation only at the lower $I_{[ZnTPPc]}$ of 2.6
nmol·cm$^{-2}$ (Figure 2C). Increasing $\Gamma_{[ZnTPPc]}$ from 2.6 to 4.0 nmol·cm$^{-2}$ significantly decreased the lag-time for H-type formation (Figure 2D). The kinetic profiles for the higher $\Gamma_{[ZnTPPc]}$ of 4.8 nmol·cm$^{-2}$ were qualitatively similar but out of sequence with the 2.6 and 4 nmol·cm$^{-2}$ profiles. This was attributed to the greater difficulty in isolating the pure spectra by PCA analysis due to the rapid enhancement in the overlapping of the spectrophotometric signals of the individual interfacial nanostructures as $\Gamma_{[ZnTPPc]}$ increased.

**Ordinary differential equations (ODE)-based kinetic modelling.** The kinetic profiles in Figure 2C-D evidence the existence of two distinct interfacial ZnTPPc nanostructures, but the details of the interconversion mechanism between the J- and H-type species was not immediately evident. Based on the MCR-ALS analysis, two mechanisms can be proposed: (i) direct conversion from J- to H-type nanostructures or (ii) via two parallel pathways where both nanostructures compete for free monomers. Although the direct conversion mechanism is intuitively attractive, recent reports have demonstrated that competitive pathways in supramolecular polymerisation are an increasingly observed phenomenon.$^{6,12,19,40–42}$

Two kinetic models based on Ordinary Differential Equations (ODE) were developed. These models are summarised in Figure 3. In Model 1, two competitive cooperative (nucleation-elongation) pathways were coupled, whereas in Model 2, an isodesmic pathway competed with a cooperative pathway. Model 1 employs 6 rate constants, while Model 2 employs 5 rate constants. Model 1 indicates that, regardless of $\Gamma_{[ZnTPPc]}$, the J-type nanostructure should present a small induction period as evident for the kinetic profile of the most dilute $\Gamma_{[ZnTPPc]}$ value of 2.6 nmol·cm$^{-2}$ (Figure 2C). In general, these kinetic models describe the rate of change of the interfacial nanostructure (or aggregate) concentration using the following ODE:

\[
\frac{d[M_i]}{dt} = k^+ [M] ([M_{i-1}] - [M_i]) + k^- ([M_{i+1}] - [M_i])
\]  

(1)

where $[M_i]$ is the concentration of a nanostructure of length $i$, and $k^+$ and $k^-$ are the association and dissociation rate constants, respectively. The first term of the equation accounts for the nanostructure growing by monomer association, while the second term accounts for the nanostructure shrinking by monomer dissociation. A detailed description of the kinetic modelling procedure and an overview of the full ODE-systems specifying the exact reaction steps involved are provided in the Supporting Information (SI).
Figure 3. Kinetic models explored to simulate porphyrin supramolecular polymerisation (or self-assembly) through two coupled pathways competing for the porphyrin monomers at the immiscible liquid|liquid interface. The kinetic models are based on monomer association and dissociation of a supramolecular polymerization consisting of two coupled cooperative (nucleation-elongation) pathways (Model 1) or an isodesmic pathway coupled with a cooperative pathway (Model 2). Models 1 and 2 employ a total of 6 and 5 rate constants, respectively, as explained in detail in the SI.

Kinetic constants for both models were extracted from the $I_{[ZnTPPc]}$ profiles for the dataset obtained at pH 5.8 using a $I_{[ZnTPPc]}$ value of 4 nmol·cm$^{-2}$, see Figure 4. At these conditions, the interfacial concentrations of both the J- and H-type nanostructures reached a stable equilibrium after 150 s. The Markov Chain Monte Carlo (MCMC) method was selected for the fitting procedure given its robust predictions based on the parameters uncertainty. This aspect is of paramount importance as the main issue affecting the resolution of bilinear data in MCR is the non-unicity of the solution due to rotational and intensity ambiguities of the
solution. The use of constraints can diminish these ambiguities, although it does not eliminate them completely. Solutions in MCR are usually represented as feasible bands.

![Image of kinetic profiles](image)

**Figure 4. Extracting the kinetic constants from the MCR-ALS analysis of interfacial ZnTPPc self-assembly.** Two models were explored (see Figure 3 and the SI) and for both models the kinetic constants were extracted from the $I_{[\text{ZnTPPc}]}$ kinetic profiles for the dataset obtained at pH 5.8 using a $I_{[\text{ZnTPPc}]}$ value of 4 nmol·cm$^{-2}$. The best-fits obtained for the time-concentration curves from (A, B) Model 1 and (C, D) Model 2 for the J- and H-type nanostructures (black lines), respectively, were compared with the kinetic profiles obtained by MCR-ALS analysis (red line). The grey areas indicate the sensitivity range based on the parameter distributions generated using the Markov Chain Monte Carlo (MCMC) method. Parameter values determined by MCMC for Models 1 and 2 are presented in Table 1. Further information regarding the parameter distribution can be found in the SI (Tables S2-S6 and Figures S7-S12).

To scale the solutions obtained by MCR-ALS, it was assumed that at equilibrium all porphyrin monomers were either as an H- or J-type nanostructure, and therefore the mass balances for Models 1 and 2 were defined as follows:

$$I_{[\text{ZnTPPc}]} = I_{[\text{H}]} + I_{[\text{J}]} \quad (2)$$

$$I_{[\text{ZnTPPc}]} = I_{[\text{H}]} + \sum I_{[\text{J}_i]} \quad (3)$$
Equations (2) and (3) correspond to Models 1 and 2, respectively. In Model 2, as the J-type nanostructure is formed through an isodesmic model, the total interfacial concentration is given by the term $i \sum T[i_f]$. The fitting process using MCMC is described in detail in the SI.

Parameter values determined by MCMC for Models 1 and 2 are presented in Table 1. Further information regarding the parameter distribution can be found in the SI (Tables S2-S6 and Figures S7-S12). For Model 1, the values clearly show that the nucleation constant ($K_n = k_1/k_2 = 2.70 \times 10^{-6}$ cm$^2$·nmol$^{-1}$) of the J-type nanostructure is four orders of magnitude larger than the nucleation constant of the H-type nanostructure ($K_n = k_4/k_5 = 2.48 \times 10^{-10}$ cm$^2$·nmol$^{-1}$). In contrast, the elongation constant of the J-type nanostructure ($K_e = k_1/k_3 = 9.96$ cm$^2$·nmol$^{-1}$) is 2.5 times smaller than the elongation constant of the H-type nanostructure ($K_e = k_4/k_6 = 24.8$ cm$^2$·nmol$^{-1}$). For Model 2, $K_n$ of the H-type nanostructure is $2.80 \times 10^{-3}$ cm$^2$·nmol$^{-1}$, while $K_e$ of the H-type nanostructure is 4.5 times bigger than $K_e$ of the J-type nanostructure ($3.15$ and $14.0$ cm$^2$·nmol$^{-1}$ for the J- and H-type, respectively).

To further investigate the parameter uncertainty found by MCMC, a sensitivity analysis was completed (see Table S5 and S6). The sensitivity coefficients as function of time for both interfacial ZnTPPc nanostructures are shown in Figure S11 and S12. Both models present similar results; in the case of the J-type nanostructure, the association constant for this aggregate ($k_1$ for both models) has a positive effect over the formation of this nanostructure. Meanwhile, the association of the H-type and dissociation of the J-type ($k_4$ and $k_2$, for model 1 and $k_3$ and $k_2$ for model 2, respectively) have a negative effect. In contrast, H-type
nanostructures present the opposite trend. These results clearly show how these pathways are competing. Additionally, Figure S11 and S12 show that the output for both models is more sensitive to the parameters when the H-type nanostructure starts to rise sharply. Finally, it is clearly seen that the sensitivity of the nucleation constant for H-type ($k_5$ and $k_4$ for Model 1 and Model 2, respectively) is small for both nanostructures. Hence, the value of this parameter can change considerably and the effect to the output is small.

The best-fittings found by MCMC, and overlaid by the MCR-ALS result (red line) for each nanostructure, are shown for Model 1 in Figure 4A-B and Model 2 in Figure 4C-D. Clearly, both models can reproduce the formation of the H-type nanostructure accurately (Figures 4B and 4D). However, for the J-type nanostructure, although the kinetic behaviour is reproduced qualitatively, the match is not perfect in either case (Figures 4A and C) and especially poorly described by Model 2 at the beginning of the process (Figure 4C).

To determine the dependence of the kinetic profiles on $I_{[ZnTPPc]}$, time-concentration curves for the J- and H-type nanostructures for both models were simulated using the values shown in Table 1 (Figure 5). Model 1 predicted a strong interfacial concentration dependence of the lag-time for the formation of the H-type nanostructure (Figure 5B). This period is reduced by more than 100 s when $I_{[ZnTPPc]}$ increased from 2 to 5 nmol·cm$^{-2}$, a range covered by our experimental data in Figure 2. In the case of the J-type nanostructure, the induction period slightly increased with the concentration. In contrast, for Model 2, the dependence of the kinetic profiles on $I_{[ZnTPPc]}$ was weak. The induction period of the H-type nanostructure changed by less than 50 s when $I_{[ZnTPPc]}$ increased from 2 to 5 nmol·cm$^{-2}$. In the same way, the kinetic profile for the J-type nanostructure was relatively unaffected. Thus, the experimentally observed dynamic behaviour found by MCR-ALS in Figure 2 was better described by Model 1: two cooperative pathways competing for the free monomers adsorbed at the liquid|liquid interface. It is worth nothing that while the current two-pathway model provides a minimal description of the experimental observations, the actual system may involve additional equilibria such as fragmentation and coagulation, or the diffusion of ZnTPPc across the interface.
Figure 5. Simulation of the $I_{[ZnTPPc]}$ kinetic profiles as a function of increasing $I_{[ZnTPPc]}$. The time-concentration curves from (A, B) Model 1 and (C, D) Model 2 for the J- and H-type nanostructures, respectively, were simulated using the parameter values determined by MCMC for each model (shown in Table 1). $I_{[ZnTPPc]}$ was varied from 2 to 5 nmol·cm$^{-2}$. The arrow indicates the direction of increasing interfacial concentration.

Modifying pathway selection to favour the formation of the metastable J-type nanostructure. According to the Hofmeister series, citrate (and its derivatives) is a kosmotropic agent.$^{49}$ Thus, in an effort to direct the pathway selection, we investigated how increasing the concentration of this supramolecular structure-stabilising molecule in the bulk aqueous phase would influence the competing pathways. The evolution of the TIR-UV/vis spectra at pH 5.8, with a $I_{[ZnTPPc]}$ of 4 nmol·cm$^{-2}$ and employing 10, 50, 100, and 250 mM citric acid concentrations in the bulk aqueous phase, respectively, are shown in Figure 6. Under these conditions, the spectral evolution differs significantly with 10 mM citric acid in the bulk aqueous phase (as shown also in Figure 1A) under otherwise identical experimental conditions. At a concentration of 50 mM citric acid (Figure 6B), the ZnTPPc monomers initially adsorbed at the liquid|liquid interface and subsequently the Soret band red-shifted, indicating the formation of a J-type nanostructure ($\lambda_{\text{max.}} = 436$ nm). Finally, a shoulder appeared centred at 418 nm and caused the main peak to blue-shift slightly by 2 nm. The latter suggests the presence
of both interfacial nanostructures, with the J-type predominant over the H-type. An analysis by PCA was performed (Figure S13). However, due to severe overlapping of the spectra, only one significant component was detected in this dataset. At citric acid concentrations ≥100 mM, the band (or shoulder) corresponding to the H-type nanostructure (λ_{max} = 418 nm) disappeared and only red-shifted spectra were observed (λ_{max} = 442 nm), see Figure 6B-C. These TIR-UV/vis spectra remained unchanged over a period of 24 hours. Thus, we concluded that at high citric acid concentrations, formation of the H-type nanostructure was completely inhibited.

**Figure 6. Modifying pathway selection to favour the formation of the metastable J-type nanostructure.** Comparison of time dependent TIR-UV/vis spectra of ZnTPPc interfacial self-assembly at the aqueous|organic interface as a function of the bulk aqueous citric acid concentration. [ZnTPPc]_aq was 8 μM, the pH was adjusted to 5.8 and the citric acid concentration was either (A) 10, (B) 50, (C) 100 or (D) 250 mM, respectively. The red spectra are that of bulk aqueous ZnTPPc at pH 5.8.

The microscopic morphologies of the films of interfacial ZnTPPc nanostructures self-assembled at pH 5.8, with a \( \Gamma_{{[\text{ZnTPPc}]}_\text{aq}} \) of 4 nmol·cm\(^{-2} \) and using either 10 or 100 mM citric acid in the bulk aqueous phase were probed ex situ using SEM and AFM (Figure 7). The influence of the citric acid concentration on the microscopic morphologies was profound, with 10 mM
citric acid leading to the H-type nanostructures predominantly and 100 mM citric acid leading to the J-type nanostructures exclusively. Both SEM (Figure 7A-B) and AFM (Figure 7C-D) images clearly show that films consisting of flakes, some of which were stacked over each other, were formed using 10 mM citric acid. By contrast, films that were largely planar and without flakes were formed using 100 mM citric acid (Figure 7E-H). Furthermore, the presence of flakes significantly increased the root-mean-square (RMS) roughness of the films formed using 10 mM citric acid compared with the planar films formed using 100 mM citric acid, as measured by AFM and summarised in Table S7.

Figure 7. Ex situ characterisation of the morphology of the interfacial ZnTPPc films by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The interfacial ZnTPPc films were prepared with either (A-D) 10 mM or (E-F) 100 mM bulk aqueous citric acid concentrations, leading to predominately H- or J-type nanostructures in the films, respectively. Otherwise the experimental conditions were identical, as described in Figure 6. (D) and (H) are AFM images recorded using semi-contact mode of the areas of the films indicated by the white rectangles in (C) and (G), respectively.

Conclusions

Our kinetic analysis of the ZnTPPc self-assembly process using TIR-UV/vis spectra obtained in situ at the liquid|liquid interface showed the presence of kinetically favoured metastable J-type nanostructures that form quickly but then transform into the thermodynamically favoured H-type nanostructures. Numerical modelling of the kinetic data suggests that both nanostructures were produced by a cooperative (nucleation-elongation) mechanism. These nanostructures formed in parallel and competed for the free monomers adsorbed at the interface. Upon confirming that spontaneous supramolecular polymerization of ZnTPPc at the liquid|liquid interface is indeed controlled by pathway complexity; we
demonstrated that varying the concentration of the kosmotropic citric acid aqueous electrolyte can change the thermodynamic preference of the assembly process. We can force aggregation completely down the kinetically favoured pathway so that, by increasing the concentration of citric acid, we obtain only metastable J-type nanostructures. We show that the morphology of the resulting interfacial films of ZnTPPc nanostructures is significantly altered by the citric acid concentration using ex situ AFM and SEM analysis. This work demonstrates that the stability of supramolecular materials can be manipulated in a controllable fashion at an immiscible liquid|liquid interface. Such pathway selection opens opportunities to rationally design optimal nanostructures from the same building-blocks with different targeted features for specific applications, such as in photovoltaic\textsuperscript{50} and molecular electronic\textsuperscript{51,52} technologies. Furthermore, the presence of competing self-assembly pathways at liquid|liquid interfaces is not restricted to porphyrins and should be readily observed in other systems, e.g., the formation of natural protein-based fibrils on membranes.\textsuperscript{53,54}

**Associated content**

**Supporting information**

Experimental and computational methods. Supplementary text detailing both models used to simulate the kinetic data, and details of Markov Chain Monte Carlo (MCMC) method. Supplementary figures and tables describing the experimental methodology, UV/vis spectroscopic studies probing the effect of pH and porphyrin concentration on the kinetics of interfacial nanostructure formation, and principal component analysis (PCA) and MCMC analysis of the kinetic datasets.

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Supporting Information

Pathway Complexity in Supramolecular Porphyrin Self-Assembly at an Immiscible Liquid|Liquid Interface

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S1. EXPERIMENTAL AND COMPUTATIONAL METHODS

**Reagents.** All chemicals were used as received without further purification. All aqueous solutions were prepared using high purity water (>18.2 MΩ-cm) from a Millipore MilliQ filtration system. α,α,α-trifluorotoluene (TFT, ≥99%), lithium hydroxide monohydrate (LiOH.H₂O, ≥98%), methyltrichlorosilane (99%) and citric acid (≥99.5%) were purchased from Sigma-Aldrich. Zinc(II) 5,10,15,20-(tetra-4-carboxyphenyl)porphyrin (ZnTPPc) was obtained from PorphyChem. Aqueous solutions of ZnTPPc were prepared by directly dissolving the solid in the lithium citrate buffer pre-adjusted to the desired pH, followed by sonication of the solution for three minutes.

**In situ UV/vis spectroscopy in total internal reflection (TIR-UV/vis).** ZnTPPc self-assembly was studied in situ at the interface between an aqueous phase containing 10 mM (analytical concentration) citric acid and a neat organic phase (TFT) by TIR-UV/vis using a custom-built optical setup (Figure S1). Spectra were obtained every 0.5 s for up to 1000 s. The self-assembly process was performed using the “Porphyrin Last” protocol,\(^1\) involving the addition of a known amount of porphyrin stock solution to the aqueous phase. The interfacial ZnTPPc concentration (\(I_{ZnTPPc}\)) was determined using the isotherm of this biphasic system reported recently.\(^2\) The pH of the aqueous phase was adjusted to values between 5.0 and 6.8 with LiOH, whereas the bulk aqueous ZnTPPc concentration ([ZnTPPc] \(_{aq}\)) was 8 μM, *i.e.*, equivalent to a \(I_{ZnTPPc}\) value of 4 nmol-cm\(^{-2}\) at pH 5.8, unless stated otherwise.

**Ex situ microscopy characterisation.** The porphyrin films were gently transferred to a silicon substrate for scanning electron microscopy (SEM) and atomic force microscopy (AFM) analysis by bringing the solid support into contact with the interface. Prior to imaging, the samples were sequentially rinsed with water and TFT. SEM was performed was performed using an SU-70 Hitachi operated at 5 kV. AFM was performed using NT-MDT’s Ntegra Spectra II. The topography was recorded using semi-contact mode. The radius of curvature of the probe tip was less than 35 nm. The resonant frequency of the probe was 134.63 kHz. The probe stiffness was 5.83 N·m\(^{-1}\). The gain of the lock-in-amplifier was set to 0.4. The scan size was set to 256 × 256 pixels. As the samples were expected to be rough in nature due to the stacking of flakes, the scan rate was 0.5 Hz.

**Multivariate Curve Resolution–Alternating Least Squares (MCR-ALS) analysis.** MCR-ALS was used to analyze the spectral evolution of interfacial ZnTPPc at the aqueous|organic interface. This tool allows the spectral evolution of individual H- and J-type
nanostructures to be separated. The calculations described herein were performed in R (the foundation for statistical computers, version 3.4.4). All UV/vis spectra measured during the self-assembly process were arranged in a data matrix $Y(r \times c)$, where rows $r$ were the spectra recorded at different times during the reaction and columns $c$ were kinetic profiles collected at different wavelengths. The raw spectra were further treated using the package baseline for smoothing and correcting the drift of the signal (Figure S2).

The decomposition of the matrix $Y$ was achieved by using MCR-ALS, a well-established and widespread decomposition method meant to solve complex mixtures without any assumptions about the composition of the system. Importantly, significant chemical information can be introduced in the optimization process under the form of constraints. MCR-ALS decomposes the matrix $Y$ according to the following equation

$$Y = CS^T + E$$  \hspace{1cm} (S1)

where matrix $S^T (n \times c)$ contains the spectral profiles of $n$ pure resolved components, matrix $C (r \times n)$ describes the concentration profiles of these $n$ species, and $E$ represents the error matrix associated with the reconstruction. The first step of the decomposition required the determination of the number of significant components in the experimental data matrix by Principal Component Analysis (PCA). PCA is a reduction tool designed to identify the amount of variance present in a certain dataset and the determination of linearly independent components. Therefore, PCA allows the rank reduction of a large dataset to a few relevant components. This tool was implemented using the package FactoMineR from R.

MCR-ALS was used according to the functions in package ALS. For MCR, only the evolution of the porphyrin Soret band was analysed in the wavelength range from 400 to 484 nm. The initial matrix $C$ was estimated by means of the detection of the purest concentration profiles with a selected level of noise of 5%. The ALS routine was run employing the following soft constraints: non-negativity and unimodality for both pure spectra and concentration profiles. The “badness” of fit of the model obtained by MCR-ALS was evaluated by determining the Lack of Fit (LOF) parameter using the following equation:

$$\%LOF = 100 \times \sqrt{\frac{\sum \sum (y_{ij} - \hat{y}_{ij})^2}{\sum \sum \hat{y}_{ij}^2}}$$  \hspace{1cm} (S2)
where \( y_{ij} \) and \( y'_{ij} \) are the experimental and calculated absorbance values, respectively. A second parameter was used to corroborate the quality of the optimization; the percentage of explained variance \((r^2)\), defined as

\[
 r^2 = 100 \times \frac{\sum_i \sum_j (y_{ij} - y'_{ij})^2}{\sum_i \sum_j y_{ij}^2} 
\]  
(S3)

S2. MODEL 1: COUPLING OF TWO COMPETITIVE COOPERATIVE (NUCLEATION-ELONGATION) PATHWAYS

The interfacial self-assembly of ZnTPPc can be explored using a one-dimensional nanostructure formation (or aggregation). This means we only consider the aggregation of a chain, and interactions with other chains in two- or three-dimensions are negligible. We modelled the size of the nanostructures by monomer association and dissociation, hence the proposed mechanism for the cooperative pathway \((J\text{-type nanostructure})\) is given by:

\[
\begin{align*}
M + M & \rightleftharpoons_{k_2}^{k_1} J_2 \\
M + J_2 & \rightleftharpoons_{k_3}^{k_1} J_3 \\
M + J_3 & \rightleftharpoons_{k_3}^{k_1} J_4 \\
M + J_4 & \rightleftharpoons_{k_3}^{k_1} J_5 \\
M + J_5 & \rightleftharpoons_{k_3}^{k_1} J_6 \\
M + J_6 & \rightleftharpoons_{k_3}^{k_1} J_p \\
M + J_p & \rightleftharpoons_{k_6}^{k_4} 2J_p
\end{align*}
\]  
(S4)

The proposed mechanism for the other cooperative pathway \((H\text{-type nanostructure})\) is given by:

\[
\begin{align*}
M + M & \rightleftharpoons_{k_5}^{k_4} H_2 \\
M + H_2 & \rightleftharpoons_{k_6}^{k_4} H_3 \\
M + H_3 & \rightleftharpoons_{k_5}^{k_4} H_4 \\
M + H_4 & \rightleftharpoons_{k_5}^{k_4} H_5 \\
M + H_5 & \rightleftharpoons_{k_6}^{k_4} H_6 \\
M + H_6 & \rightleftharpoons_{k_6}^{k_4} H_p
\end{align*}
\]
Here, \( M \) is the monomeric ZnTPPc adsorbed at the immiscible liquid|liquid interface, and \( J_i \) and \( H_i \) are the J- and H-type nanostructures, respectively. These nanostructures consist of \( i \) monomers. Nanostructures below a nucleus size \( n \) grow with a nucleation equilibrium constant \( K_n \), described by the kinetic constants \( k_1, k_2, k_4 \) and \( k_5 \). The association constants are \( k_1 \) and \( k_4 \) and the dissociation constants are \( k_2 \) and \( k_5 \). Meanwhile, nanostructures equal to, or above, the nucleus size grow with an elongation equilibrium constant \( K_e \), described by the kinetics constants \( k_1, k_3, k_4 \) and \( k_6 \). For cooperative polymerization, we have the following condition, \( K_n < K_e \).

To reduce the computational cost, the length of the polymers was set to 7. Further increasing the size of the chain did not affect the output. In the cooperative pathway, the last step is an autocatalytic process and therefore, to fulfil the mass balance, step 6 is irreversible following the model of Frieden for the polymerization of actin.\(^{11}\)

This mechanism produced a set of 13 ordinary differential equations (ODEs) that must be solved simultaneously.

\[
\frac{dJ_2}{dt} = k_1([M]^2 - [M][J_3]) - k_2[J_2] + k_3[J_3]
\]

\[
\frac{dJ_i}{dt} = k_1[M]([J_{i-1}] - [J_i]) + k_3([J_{i+1}] - [J_i]) \quad 3 \leq i \leq 5
\]

\[
\frac{dJ_6}{dt} = k_1[M]([J_5] - [J_6]) + k_3[J_6]
\]

\[
\frac{dJ_p}{dt} = k_1[M]([J_6] - [J_p]) + k_3[J_6]
\]

\[
\frac{dH_2}{dt} = k_4([M]^2 - [M][H_3]) - k_5[H_2] + k_6[H_3]
\]

\[
\frac{dH_i}{dt} = k_4[M]([H_{i-1}] - [H_i]) + k_6([H_{i+1}] - [H_i]) \quad 3 \leq i \leq 5
\]

\[
\frac{dH_6}{dt} = k_4[M]([H_5] - [H_6]) + k_6[H_6]
\]

\[
\frac{dH_p}{dt} = k_4[M]([H_6] - [H_p]) + k_6[H_6]
\]
\[
\frac{dM}{dt} = 2(k_2[J_2] - k_1[M]_2) - k_1[M] \sum_{i=3}^{7} [J_i] + k_3 \sum_{i=3}^{6} [J_i] + k_3 [J_p]^2 + 2(k_4[H_2] - k_5[M]_2) - k_4[M] \sum_{j=3}^{7} [H_j] + k_6 \sum_{j=3}^{6} [H_j] + k_6 [H_p]^2
\]

(S6)

The initial conditions (numbers at \( t = 0 \)) are as follows:

\[
[M](0) = M_0
\]
\[
[J_i](0) = 0
\]
\[
[H_j](0) = 0
\]

(S7)

The following constraints are applied:

\[
k_3[J_p] = 0.0
\]
\[
k_6[H_p] = 0.0
\]
\[
k_1/k_2 > 1
\]
\[
k_3/k_5 > 1
\]
\[
k_3/k_4 < 1
\]

(S8)

The mass balance of the reaction is:

\[
M_0 = M + i \sum_{i=2}^{n} J_i + J_p + j \sum_{j=2}^{6} H_j + H_p
\]

(S9)

Finally, the signals that are observed are assumed to originate from ZnTPPc in the monomeric state, in the J-type nanostructure and in the H-type nanostructure, the concentrations of which are given by \([J_{agg}]\) and \([H_{agg}]\):

\[
[J_{agg}] = [J_p]
\]
\[
[H_{agg}] = [H_p]
\]

(S10)

The corresponding system of coupled ODEs is solved using the functions provided by the \texttt{deSolve} package in \texttt{R}.\textsuperscript{12} An interactive version of this model can be found in the following link \url{https://entropia88.shinyapps.io/Shiny/}
S3. MODEL 2: COUPLING OF COMPETITIVE ISODESMIC AND COOPERATIVE PATHWAYS

In this model, the interfacial self-assembly of ZnTPPc is explained by means of isodesmic and cooperative pathways. The *isodesmic pathway* is given by:

\[
\begin{align*}
M + M & \rightleftharpoons \frac{k_1}{k_2} J_2 \\
M + J_2 & \rightleftharpoons \frac{k_1}{k_2} J_3 \\
M + J_3 & \rightleftharpoons \frac{k_1}{k_2} J_4 \\
M + J_4 & \rightleftharpoons \frac{k_1}{k_2} J_5 \\
M + J_5 & \rightleftharpoons \frac{k_1}{k_2} J_6 \\
M + J_6 & \rightleftharpoons \frac{k_1}{k_2} J_7
\end{align*}
\]

(S11)

The *cooperative pathway* is given by:

\[
\begin{align*}
M + M & \rightleftharpoons \frac{k_3}{k_4} H_2 \\
M + H_2 & \rightleftharpoons \frac{k_3}{k_4} H_3 \\
M + H_3 & \rightleftharpoons \frac{k_3}{k_5} H_4 \\
M + H_4 & \rightleftharpoons \frac{k_3}{k_5} H_5 \\
M + H_5 & \rightleftharpoons \frac{k_3}{k_5} H_6 \\
M + H_6 & \rightleftharpoons \frac{k_3}{k_5} H_p \\
M + H_p & \rightleftharpoons \frac{k_3}{k_5} 2H_p
\end{align*}
\]

(S12)

Here, \(M\) is the monomeric ZnTPPc adsorbed at the immiscible liquid|liquid interface, and \(J_i\) and \(H_i\) are the J-type and H-type nanostructures, respectively. These nanostructures consist of \(i\) monomers. For the isodesmic pathway, the association and dissociation constants are \(k_1\) and \(k_2\), respectively. For the cooperative pathway, nanostructures below a nucleus size \(n\) grow with a nucleation equilibrium constant \(K_n\), described by the kinetic constants \(k_3\) and \(k_4\) for...
the association and dissociation, respectively. Meanwhile, nanostructures equal to or above the nucleus size grow with an elongation equilibrium constant $K_e$, described by the kinetic constants $k_3$ and $k_5$. For cooperative polymerization, $K_n < K_e$.

To reduce the computational cost, the length of the polymers was set to 7. Further increases of the size of the chain did not affect the output. In the cooperative pathway, the last step is an autocatalytic process. Therefore, to fulfil the mass balance, step 6 is irreversible, following the model of Frieden for polymerization of actin.\(^1\)

This mechanism produced a set of 13 ODEs that must be solved simultaneously.

\[
\frac{dJ_i}{dt} = k_1[M](J_{i-1}) - J_i) + k_2(J_{i+1}) - J_i) \quad i \geq 2
\]

\[
\frac{dJ_n}{dt} = k_1[M][J_{n-1}] + k_2[J_n]
\]

\[
\frac{dH_2}{dt} = k_3([M]^2 - [M][H_3]) - k_4[H_2] + k_5[H_5]
\]

\[
\frac{dH_i}{dt} = k_3[M](H_{i-1}) - [H_i) + k_5([H_{i+1}] - [H_i]) \quad 3 \leq i \leq 5
\]

\[
\frac{dH_6}{dt} = k_3[M][H_5] - [H_6]) + k_5[H_6]
\]

\[
\frac{dH_p}{dt} = k_3[M][H_6] - [H_p]) + k_5[H_6]
\]

The initial conditions (numbers at $t = 0$) are as follows:

\[
[M](0) = M_0
\]

\[
[J_i](0) = 0
\]

\[
[H_j](0) = 0
\]

The following constraints are applied:

\[
k_5[H_p] = 0.0
\]

\[
k_1/k_2 > 1
\]

\[
k_3/k_5 > 1
\]
\[ k_3/k_4 < 1 \]  
(S15)

The mass balance of the reaction is:

\[ M_0 = M + i \sum_{i=2}^{n} J_i + j \sum_{j=2}^{6} H_j + H_p \]  
(S16)

Finally, the signals that are observed are assumed to originate from ZnTPPc in the monomeric state, in the J-type nanostructure and in the H-type nanostructure, the concentrations of which are given by \([J_{agg}]\) and \([H_{agg}]\):

\[ [J_{agg}] = i \sum_{i=2}^{n} [J_i] \]

\[ [H_{agg}] = [H_p] \]  
(S17)

The corresponding system of coupled ODEs is solved using the functions provided by the \texttt{deSolve} package in R. An interactive version of this model can be found at the following link:  
https://entropia88.shinyapps.io/Shiny/

**S4. THE MARKOV CHAIN MONTE CARLO (MCMC) FITTING PROCEDURE**

The main issue affecting the resolution of bilinear data in MCR is the non-unicity of the solution, due to rotational and intensity ambiguities of the solution. Intensity ambiguities are related to scaling issues and can be solved by the normalization of concentration profiles or resolved spectra. Rotational ambiguities are related to changes in the shape of the sought profiles, and this type of ambiguity is the most problematic for robustness and interpretation of MCR results. The use of constraints can diminish the rotational ambiguity, but not eliminate it completely. Consequently, solutions in MCR are usually represented as feasible bands, that is the space of possible solutions.

A regular nonlinear fitting procedure is not the most accurate algorithm considering these feasible bands. Thus, it is important to provide an estimate of the parameter uncertainty, and to quantify the effect of that uncertainty on the observed variables. For this purpose, the
method chosen was Markov Chain Monte Carlo (MCMC). MCMC was implemented using the package **FME**. FME uses MCMC with the Delayed Rejection and Adaptive Metropolis procedure. MCMC is an efficient sampling method where the selection of the next parameter combination depends on the last parameter set and the resulting deviation between the model and the observation. Therefore, sampling is concentrated in the region with high likelihood. This makes the method more efficient.

To avoid ‘burn-in’, the algorithm was started with the optimal parameter set as returned from the nonlinear fitting algorithm provided by the package **FME**. MCMC was run with a number of 7000 steps, a delayed reaction of 2, the number of iterations after which the parameter covariance matrix is evaluated was set to 100, and a low variance weight was given to the prior distribution compared to the posterior distribution (wvar = 0.1). Additionally, the lower and upper bounds of the parameters were carefully selected to fulfil the conditions (S5) and (S12) in Models 1 and 2, respectively.

**S5. SUPPLEMENTARY FIGURES**

**Figure S1. Experimental methodologies.** (A) Schematic representation of the setup for TIR-UV/vis absorbance measurements in TIR mode. (1) Xe light source, (2) plano-convex lenses, (3) Xe light source, (4) plano-convex lenses, (5) Xe light source, (6) Xe light source. (B) Water, Trifluorotoluene. [ZnPor] = 8 μM, [Li₂H₂Cit] = 5 mM, pH = 5.0-6.8. (C) ZnTPPC stock solution, injection, stir.
(3) infrared filter, (4) iris diaphragm, (5) mirror, and (6) Ocean Optics Maya2000 Pro spectrometer. The angle of incidence was set to 80° to ensure TIR conditions. (B) Schematic description of the biphasic system used during this work. The vertical double line represents the polarisable interface between the organic solvent α,α,α-trifluorotoluene (TFT) and the aqueous phase. (C) Simple depiction of the mixture protocol using the “Porphyrin Last” procedure. All vials were previously silanized by adding some drops of methyltrichlorosilane into the organic phase of the biphasic system. Once the interface is flat, the reaction was stopped by emptying the vials and washing them extensively with acetone.

Figure S2. Baseline correction of the TIR-UV/vis spectra. (A) Example of unprocessed spectral data which clearly shows the drift of the signal. The raw spectra were treated (B) using R (version 4.0.3) with the function `baseline.rfbaseline` for a Robust Baseline Estimation (a function that is included in the package baseline).
ZnTPPc self-assembly at an immiscible aqueous|organic interface is very sensitive to the pH of the aqueous phase.\textsuperscript{2,16} Above the $pK_a$ value of the carboxyl group on the porphyrin ($pK_a = 5.8$),\textsuperscript{9} the carboxyl groups are primarily deprotonated and the ZnTPPc monomers are negatively charged. Thus, the adsorbed monomers at the interface repel each other electrostatically and interfacial nanostructure self-assembly is inhibited. Consequently, only a band centred at 430 nm is detected in Figures S3D-F, corresponding to the Soret band of ZnTPPc monomers adsorbed at the interface. Below the $pK_a$, the ZnTPPc molecules aggregate uncontrollably in the bulk aqueous phase (red lines, Figure S1A-B). Consequently, the quantity of free monomers adsorbed at the interface is very limited, and interfacial nanostructure formation does not take place. Only at a pH value equals to the $pK_a$ of the carboxyl groups can interfacial ZnTPPc self-assembly proceed (Figure S3C).

**Figure S3. The influence of the aqueous phase pH on ZnTPPc interfacial self-assembly.** Comparison of time dependent TIR-UV/vis spectra of ZnTPPc interfacial self-assembly when the aqueous phase pH was adjusted to (A) 5.0, (B) 5.5, (C) 5.8, (D) 6.1, (E) 6.5 and (F) 6.8. All other biphasic experimental conditions were identical (bulk aqueous ZnTPPc concentration of 8 $\mu$M, 10 mM citric acid aqueous electrolyte, and the organic phase was neat TFT). TIR-UV/vis spectra were taken every 0.5 s for 500 seconds. The red spectra are that of bulk aqueous ZnTPPc at each pH.
Below 5 μM [ZnTPPc]_{aq}, the interfacial self-assembly process did not proceed, and only the Soret band attributed to adsorbed monomers at the aqueous|organic interface were detected (Figure S4A-B). For [ZnTPPc]_{aq} values between 5 and 10 μM, interfacial nanostructure formation proceeded, and all experimental datasets presented the same dynamic behaviour (Figure S4C-E). This dynamic behaviour is discussed in detail in the main text and illustrated in Figure 1C.

**Figure S4. The influence of the porphyrin concentration on ZnTPPc interfacial self-assembly.** Comparison of time dependent TIR-UV/vis spectra of ZnTPPc interfacial self-assembly when the bulk aqueous ZnTPPc concentration ([ZnTPPc]_{aq}) was adjusted to (A) 1.0, (B) 2.0, (C) 5.0, (D) 8.0, and (E) 10.0 μM. All other biphasic experimental conditions were identical (aqueous pH of 5.8, 10 mM citric acid aqueous electrolyte, and the organic phase was neat TFT). TIR-UV/vis spectra were taken every 0.5 s for 500 seconds. The red spectra are that of bulk aqueous ZnTPPc at pH 5.8.
Figure S5A reports the scree plot for the TIR-UV/vis dataset in Figure 1A (main text; $I_{[ZnTPPc]}$ of 4 nmol·cm$^{-2}$). Eigenvalues associated to each component show an elbow in the second component, marking the separation between physiochemically meaningful components and noise-related ones. This result is confirmed by analyzing the scores and loadings from the PCA in Figure S5B-C. The scores and loadings appear noisier and unstructured from the third component. Based on these considerations, we ran the subsequent MCR-ALS analysis using two principal components (PCs, see Figure 2, main text). PC$_1$ was associated to an H-type nanostructure ($\lambda_{\text{max.}} = 418$ nm) and the PC$_2$ to a J-type nanostructure ($\lambda_{\text{max.}} = 442$ nm). The porphyrin monomers adsorbed at the interface were not a significant component that contributed to the variance of the dataset. Similar results were found for the other experimental datasets at $I_{[ZnTPPc]}$ values of 0.4 and 4.8 nmol·cm$^{-2}$ (data not shown).

Figure S5. Scree plot and representation of the PCA results for the TIR-UV/vis dataset reported in Figure 1A (main text). (A) Scree plot, (B) PCA scores as function of time and (C) PCA loadings as a function of wavelength.
MCR successfully separated the original spectral data matrix into the two types of nanostructures (Figure 2 main text and Figure S6). Based on the pure spectra obtained, one spectrum was assigned to a J-type nanostructure ($\lambda_{\text{max.}} = 442$ nm) and the other to a H-type nanostructure ($\lambda_{\text{max.}} = 418$ nm). Table S1 displays the quality parameters of the modelling, in all cases the % Lack of Fit (LOF) was close or below 5%, and the percentage of explained variance ($r^2$) was greater than 97%.

Figure S6. Multivariate Curve Resolution–Alternating Least Squares (MCR-ALS) analysis of the kinetics of interfacial ZnTPPc self-assembly. (A, B) MCR-ALS resolved the pure spectra of the H- and J-type nanostructures for interfacial ZnTPPc concentration ($\Gamma_{\text{ZnTPPc}}$) values of (A) 0.4 and (B) 4.8 nmol·cm$^{-2}$ at pH 5.8, respectively. (C, D) The corresponding kinetic profiles for the H- and J-type nanostructures, $\Gamma_{\text{ZnTPPc}}$ values of (C) 0.4 and (D) 4.8 nmol·cm$^{-2}$, respectively.

Table S1. Quality control parameters extracted from MCR-ALS algorithms for each dataset.

| $\Gamma_{\text{ZnTPPc}}$ (nmol·cm$^{-2}$) | %LOF | Root sum squared (RSS) | $r^2$ |
|----------------------------------------|-------|------------------------|-------|
| 2.6                                    | 1.97  | 0.2197                 | 99.9  |
| 4.0                                    | 6.37  | 4.1581                 | 97.2  |
| 4.8                                    | 2.56  | 2.3812                 | 97.0  |
Table S2 shows the number of accepted and rejected steps for both models. Figures S7 and S8 show the traces of the MCMC chain (grey line) along the iterations for every parameter and the residuals. It is clearly seen that the chain converged because there is no apparent drift in each of the traces.

Table S2. Number of accepted and rejected steps, and number of iterations the covariance was updated for the MCMC run.

|       | Accepted steps | Rejected steps | Covariance update |
|-------|----------------|----------------|-------------------|
| Model 1 | 3924           | 4043           | 58                |
| Model 2 | 3299           | 4722           | 67                |

Figure S7. MCMC traces of the parameters from kinetic Model 1. SSR calculated is 1383.38.
Figure S8. MCMC traces of the parameters from kinetic Model 2. SSR calculated is 1468.58.
The parameter distributions found by MCMC are plotted in Figures S9 and S10, and tabulated in Table S3 and S4, for Models 1 and 2, respectively. The dissociation constants of the nuclei for both models had a non-normal distribution, meaning that their values could change over a wide range, and this had no significant effect to the output. Hence, the uncertainty for these parameters was large. By contrast, the other parameters were normally distributed except for $k_1$ and $k_3$ in Model 2 which seem to have exponential distribution shapes. The latter may be due to the lower bound restriction imposed on these parameters to fulfil condition (S12).

**Figure S9.** Histogram of the parameter distributions for kinetic Model 1.
Table S3. Parameter distributions obtained from the MCMC for kinetic Model 1.

| Parameter | $k_1$ (cm$^2$·nmol$^{-1}$·s$^{-1}$) | $k_2$ (s$^{-1}$) | $k_3$ (s$^{-1}$) | $k_4$ (cm$^2$·nmol$^{-1}$·s$^{-1}$) | $k_5$ (s$^{-1}$) | $k_6$ (s$^{-1}$) |
|-----------|----------------------------------|-----------------|-----------------|----------------------------------|-----------------|-----------------|
| mean      | 2.809×10$^{-1}$                  | 2.27×10$^4$     | 2.973×10$^{-2}$ | 2.571×10$^1$                     | 4.45×10$^8$     | 1.124×10$^{-2}$ |
| σ         | 2.612×10$^{-2}$                  | 2.04×10$^4$     | 5.190×10$^{-2}$ | 3.330×10$^{-2}$                  | 3.80×10$^8$     | 1.336×10$^{-3}$ |
| Minimum value | 2.235×10$^{-1}$                  | 4.92×10$^3$     | 1.842×10$^{-2}$ | 1.771×10$^{-1}$                  | 2.42×10$^7$     | 7.676×10$^{-3}$ |
| Maximum value | 3.976×10$^{-1}$                  | 9.86×10$^4$     | 4.949×10$^{-2}$ | 3.444×10$^{-1}$                  | 1.32×10$^9$     | 1.499×10$^{-2}$ |
| Q1        | 2.642×10$^{-1}$                  | 7.61×10$^4$     | 2.600×10$^{-2}$ | 2.331×10$^{-1}$                  | 9.49×10$^7$     | 1.040×10$^{-2}$ |
| Q2        | 2.800×10$^{-1}$                  | 1.45×10$^4$     | 2.899×10$^{-2}$ | 2.521×10$^{-1}$                  | 3.02×10$^8$     | 1.111×10$^{-2}$ |
| Q3        | 2.979×10$^{-1}$                  | 3.05×10$^4$     | 3.289×10$^{-2}$ | 2.735×10$^{-1}$                  | 8.70×10$^3$     | 1.224×10$^{-2}$ |

Figure S10. Histogram of the parameter distributions for kinetic Model 2.
Table S4. Parameter distribution obtained from the MCMC for kinetic Model 2.

| Parameter | $k_1$ (cm²·nmol⁻¹·s⁻¹) | $k_2$ (s⁻¹) | $k_3$ (cm²·nmol⁻¹·s⁻¹) | $k_4$ (s⁻¹) | $k_5$ (s⁻¹) |
|-----------|-----------------|-------------|-----------------|-------------|-------------|
| mean      | 3.482           | 6.89×10⁻¹   | 7.73×10⁻¹       | 8.65×10²    | 3.700×10⁻²  |
| σ         | 1.024           | 2.06×10⁻¹   | 3.96×10⁻¹       | 1.61×10³    | 3.926×10⁻³  |
| Minimum   |                 |             |                 |             |             |
| value     | 2.718           | 2.06×10⁻¹   | 4.72×10⁻¹       | 1.48×10²    | 2.944×10⁻²  |
| Maximum   |                 |             |                 |             |             |
| value     | 8.706           | 1.00        | 2.91            | 2.12×10⁴    | 5.252×10⁻²  |
| Q₁        | 2.875           | 5.23×10⁻¹   | 5.31×10⁻¹       | 1.98×10²    | 3.440×10⁻²  |
| Q₂        | 3.126           | 7.35×10⁻¹   | 6.13×10⁻¹       | 3.12×10²    | 3.600×10⁻²  |
| Q₃        | 3.570           | 8.55×10⁻¹   | 8.33×10⁻¹       | 7.64×10²    | 3.88×10⁻²   |
To further investigate the parameter uncertainty found by MCMC, a sensitivity analysis was done. Local sensitivity analysis was performed according to the function provided by the package FME. A matrix, $S_{ij}$, that contained the normalised and dimensionless sensitivities, was generated and every element of this matrix was defined as

$$\bar{S}_{ij} = \frac{k_j}{y_i} \frac{\partial y_i}{\partial k_j}$$ (S18)

where $y_i$ is an output variable, and $k_j$ is the $j$th parameter. These coefficients were normalized by the nominal value of $y_i$ and $k_j$. The higher the absolute sensitivity value, the more important the parameter. Thus, the magnitudes of the sensitivity can be used to rank the importance of the parameter to the output variable.

A summary of these ranks for Models 1 and 2 are presented in Tables S5 and S6. Here, $L_1$ and $L_2$ are defined as follows:

$$L_1 = \sum \frac{|S_{ij}|}{n}$$ (S19)

$$L_2 = \sqrt{\frac{\sum S_{ij}^2}{n}}$$ (S20)

**Table S5.** Normalized sensitivity coefficients for kinetic Model 1.

| Value | $L_1$ | $L_2$ | Mean | Min | Max |
|-------|-------|-------|------|-----|-----|
| $k_1$ | 0.267 | 3.20  | 5.97 | -1.82 | -17.11 | 10.48 |
| $k_2$ | 9.87×10$^4$ | 0.31  | 0.66 | 0.210 | -1.18 | 2.26 |
| $k_3$ | 2.68×10$^{-2}$ | 1.24  | 2.12 | 0.45  | -1.98 | 24.20 |
| $k_4$ | 0.278 | 3.57  | 6.79 | 2.39  | -8.31 | 19.60 |
| $k_5$ | 1.12×10$^{-9}$ | 0.09  | 0.25 | -0.04 | -1.12 | 0.37 |
| $k_6$ | 1.12×10$^{-2}$ | 0.28  | 0.37 | 0.09  | -0.31 | 1.10 |

**Table S6.** Normalized sensitivity coefficients for kinetic Model 2.

| Value | $L_1$ | $L_2$ | Mean | Min | Max |
|-------|-------|-------|------|-----|-----|
| $k_1$ | 1.80  | 2.24  | 3.82 | -1.28 | -12.4 | 2.32 |
| $k_2$ | 2.20×10$^{-1}$ | 2.01  | 3.35 | 1.04  | -2.33 | 11.1 |
| $k_3$ | 1.10  | 3.08  | 5.39 | 1.91  | -3.02 | 17.07 |
| $k_4$ | 5.20×10$^{-1}$ | 0.36  | 0.77 | -0.32 | -1.98 | 0.25 |
| $k_5$ | 4.30×10$^{-2}$ | 0.6   | 0.76 | 0.12  | -0.71 | 1.34 |
Figure S11. Sensitivity coefficients as a function of time for kinetic Model 1 for (A) the J-type nanostructure and (B) the H-type nanostructure.

Figure S12. Sensitivity coefficients as a function of time for kinetic Model 2 for (A) the J-type nanostructure and (B) the H-type nanostructure.
PCA was applied in order to determine the number of significant components in the dataset presented in Figure 6B (main text) where the aqueous citric acid concentration is set at 50 mM. From the scree plot in Figure S13A, the eigenvalues associated to the first component explain more than 97% of data variance. This result is further confirmed by analyzing the scores and loadings from the PCA (Figure S13B-C). Although, from a simple inspection of Figure 6B the presence of more than one species is clearly discerned, the overlapping is so severe that a curve resolution will not successfully resolve the data. Based on these considerations, no MCR-ALS was performed on the datasets in Figure 6 involving large concentrations (50, 100 and 250 mM) of aqueous citric acid electrolyte.

Figure S13. Scree plot and representation of the PCA results for the TIR-UV/vis dataset reported in Figure 6B (main text). (A) Scree plot, (B) PCA scores as function of time and (C) PCA loadings as a function of wavelength.
The microscopic morphology of the interfacial nanostructures using 10 and 100 mM aqueous citric acid electrolyte was probed using AFM (Figures 7C-D and 7G-H, main text). AFM survey scans in semi-contact mode of 10 \( \mu \text{m} \times 10 \mu \text{m} \) were initially performed to investigate the surface roughness and optimise the scan parameters. The measured RMS and \( R_a \) roughness values of the H-type nanostructures (when using 10 mM citric acid) were 32.10 and 23.61 nm, respectively. The measured RMS and \( R_a \) roughness values of the J-type nanostructures (when using 100 mM citric acid) were 5.97 and 4.35 nm, respectively.

Lower resolution scans of 0.5 \( \mu \text{m} \times 0.5 \mu \text{m} \) and 1.0 x 1.0 \( \mu \text{m} \) were performed on areas of interest in Figures 7D and 7H, respectively. Here, for the H-type nanostructures, the RMS and \( R_a \) values were 9.12 and 5.38 nm. The high roughness is due to the scan taking place over the edge of a flake. The J-type nanostructures had RMS and \( R_a \) values of 0.73 and 0.59 nm. The reduced roughness of the J-type nanostructures is due to the apparent absence of flakes. This information is summarized in Table S7. These results agree with the images obtained by SEM (Figures 7A-B and 7E-F, main text).

**Table S7. Summary of AFM parameters**

| Sample          | H-type nanostructures | J-type nanostructures |
|-----------------|-----------------------|-----------------------|
| Area (\( \mu \text{m} \times \mu \text{m} \)) | 5.0 x 3.5 | 0.5 x 0.5 | 5.0 x 5.0 | 1.0 x 1.0 |
| RMS (nm)        | 32.01 | 9.12 | 5.97 | 0.73 |
| \( R_a \) (nm)  | 23.61 | 5.38 | 4.35 | 0.59 |
S5. SUPPLEMENTARY REFERENCES

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