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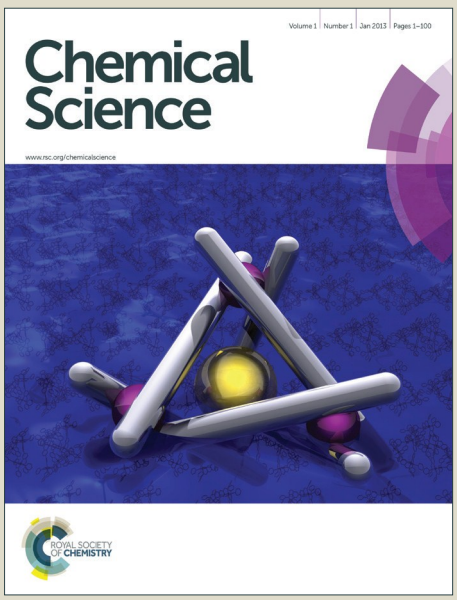

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ARTICLE

Multivalence cooperativity leading to "all-or-nothing" assembly: the case of nucleation-growth in supramolecular polymers.

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All-or-nothing molecular assembly events, essential for the efficient regulation of living systems at the molecular level, are emerging properties of complex chemical systems that are largely attributed to the cooperativity of the weak interactions. The link between the self-assembly and the interactions responsible for the assembly is however often poorly defined. In this work we demonstrate how the chelate effect (multivalence cooperativity) can play a central role in the regulation of the all-or-nothing assembly of structures (supramolecular polymers here), even if the building blocks are not multivalent. We have studied the formation of double-stranded supramolecular polymers formed of Co-metalloporhyrin and bi-pyridine building blocks. Their cooperative nucleation-elongation assembly can be summarized as a thermodynamic cycle, where the monomer weakly oligomerizes linearly or weakly dimerizes laterally. But thanks to the chelate effect, the lateral dimer readily oligomerizes linearly and the oligomer readily dimerizes laterally, leading to long double stranded polymers. A model based on this simple thermodynamic cycle can be applied to the assembly of polymers with any number of strands, and allows determining the length of the polymer and the all-or-nothing switching concentration, from the pairwise binding constants. The model, which is consistent with the behaviour of supramolecular polymers such as microtubules and gelators, clearly shows that all-or-nothing assembly is triggered by a change in the mode of assembly, from non-multivalent to multivalent, when a critical concentration is reached. We believe this model is applicable to many molecular assembly processes, ranging from the formation of cell-cell focal adhesion points to crystallization.

Introduction

The regulation of biological systems at the molecular level requires that molecular assembly events respond efficiently to changes in the environment. This responsiveness often rests on the amplification of an input signal (for example, a change in temperature, or the presence of a particular chemical), that leads to an all-or-nothing switch between assembled and de-assembled states. The amplification of the input signal is an emerging property of the system, and can be attributed to the positive cooperativity between the intermolecular interactions involved, resulting in increased stability (above the simple addition of all the pairwise interactions) of assemblies held by multiple interactions.[1,2] Cooperativity can be classified in allosteric cooperativity and chelate cooperativity, also termed multivalence cooperativity. In the first, initial binding events lead to changes in the assembled molecules (such as polarization of H-bond donors or conformational changes) that make the binding of additional molecules more efficient. In chelate cooperativity, the formation of a first interaction between two molecules pays the entropic cost of bringing the molecules together, making any subsequent interaction more stable.[3-5]

The linear assembly of molecules leads to the formation of supramolecular polymers, which in biomolecular systems are involved in many vital cellular processes, including cell division (microtubules) or cell motion (actin filaments).[6] Supramolecular polymers are also of interest for technological and biomedical applications.[7,8] For example, supramolecular polymers produced by the self-assembly of small synthetic molecules have properties that are tuneable by using different components and are interesting for the development of optoelectronic devices.[9-11] The crosslinking of nano-filaments leads to the formation of small molecule based gels, with applications ranging from drug delivery to tissue engineering.[12-18]

In supramolecular polymerization, especially in biological examples such as microtubules, assembly of the building blocks may take place in a cooperative fashion, in what is termed nucleation-elongation mechanism. This cooperative effect can be quantified,[19,20] and has been often explained in terms of allosteric cooperativity, whereby after a few molecules form the nucleus, conformational changes take

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Electronic Supplementary Information (ESI) available: Experimental methods, including sample preparation for NMR, UV and Electron Microscopy experiments, building up of the X-Ray derived dimer structure, analysis of binding of monovalent ligands, additional data on binding constant determination, effect of buffer concentration in polymer dimerization and detailed derivation of the equations. See DOI: 10.1039/x0xx00000x

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place that enhance the interaction of subsequent molecules.[21-24]

The chelate effect, normally seen as a property of complexes formed between two molecules held together by multiple interactions, has not been attributed an explicit role in the assembly of supramolecular polymers, except for the special case of helical polymers.[19,25,26] Yet, in most cases, supramolecular polymers are formed by bundles of several strands. Within such structures, the assembly can be interpreted as the combination of two molecular recognition processes: the longitudinal growth of each strand and the lateral assembly between the strands. The latter involves the simultaneous lateral interaction of many building blocks and it is therefore subjected to a multivalent cooperativity effect. In other words, the nucleation step would yield a multivalent nucleus, resulting in the amplification of the polymerization process thanks to the chelate effect. Here we study the assembly of a polymer formed of two different building blocks: a cobalt metalloporphyrin and 4,4'-bipyridine. Porphyrin-containing assemblies are intrinsically interesting as they have potential applications for light-harvesting and the development of nano-wires.[27-29] In the present work we take advantage of the favourable spectroscopic properties of the porphyrins[30,31] to carry out a detailed analysis of the role played by the chelate effect in supramolecular polymerization.

Results and discussion

Co metalloporphyrin **C** was prepared by metalation of free-base with cobalt acetate.[32] The sharp ^{1}H-NMR signals show that the metal center in **C** is diamagnetic Co(III) rather than paramagnetic Co(II) . The ^{1}H-NMR spectrum of porphyrin **C** in aqueous buffers (sodium phosphate, 5 to 100 mM, pH 7.2) is independent of the concentration up to 3 mM tested in our experiments, consistent with the absence of self-aggregation in these conditions. The addition of di-valent bipyridine (**B**) to di-valent porphyrin **C** at concentrations between 25 and 200 μM leads to changes in the ^{1}H-NMR spectrum that are consistent with the formation of small oligomeric species with alternated **CB** building blocks (Fig. 1). Diffusion NMR experiments, together with the changes on ^{1}H-NMR signal intensity with changing **C**/**B** ratios, allowed identifying the presence of complexes **CB**, **CB$_2$**, **C$_2$B**, **C$_2$B$_2$** and **C$_2$B$_3$** (Fig. 2A) (see Supplementary Information, Fig. S1 and Table S1 for details).[33] K_1, K_2 and K_3 are the stepwise binding constants for the formation of **CB**, **CB$_2$** and **C$_2$B** , i.e.

$$K_1 = \frac{[\mathbf{CB}]}{[\mathbf{C}][\mathbf{B}]} \quad (1)$$

$$K_2 = \frac{[\mathbf{CB_2}]}{[\mathbf{CB}][\mathbf{B}]} \quad (2)$$

$$K_3 = \frac{[\mathbf{C_2B}]}{[\mathbf{CB}][\mathbf{C}]} \quad (3)$$

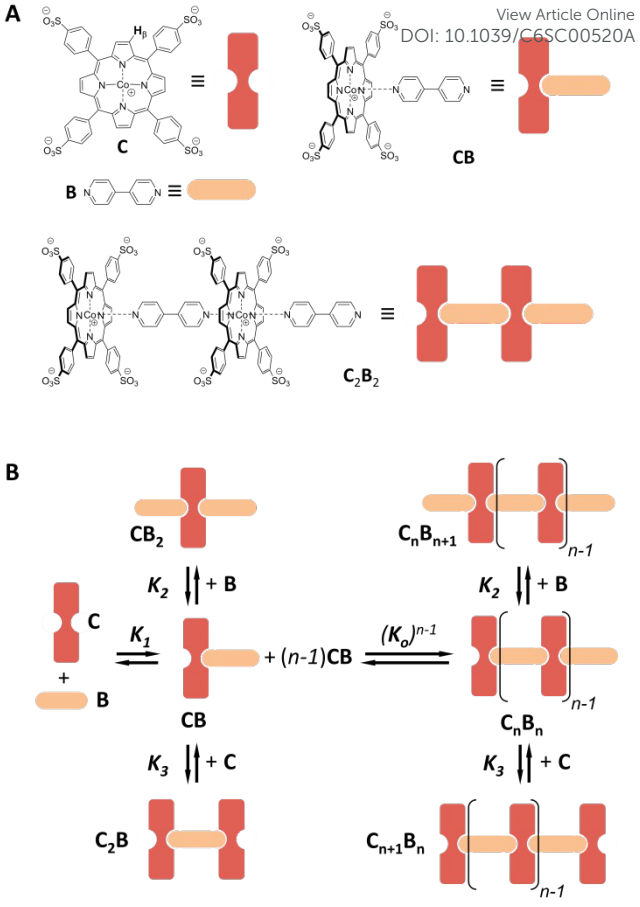

Figure 1. A. Chemical structure of **C** and **B** and of choice complexes, together with their cartoon representations. The β hydrogen of the **C** (whose ^{1}H-NMR signal is followed in the NMR experiments) is highlighted. B. Schematic representation of the oligomerization equilibria.

K_1, K_2 and K_3 were determined by integration of the corresponding ^{1}H-NMR peaks. K_1 and K_2 calculated by UV titration are in agreement with the values derived from NMR data (Fig. S2, Table 1). All binding events involving **C** and **B** can be expressed as a function of K_1, K_2 and K_3 . For example, the oligomerization constant of **CB** repeats can be written as (Fig. 1B, see Supplementary Information for details):

$$K_o = \frac{[\mathbf{C}_n\mathbf{B}_n]}{[\mathbf{C}_{n-1}\mathbf{B}_{n-1}][\mathbf{CB}]} = \frac{4(K_2K_3)}{K_1} \quad (4)$$

Equations (1)-(4) in combination with the mass balance allow modelling the distribution of species observed in the NMR experiments (Fig. 2A and 2B). For a mixture of **C** and **B** in a 1 to 1 ratio, the length of the oligomer can be expressed by the average number of **CB** repeats <*N*> , calculated as follows (see Supplementary Information for details):

$$< N >= \frac{1}{1-4K_2K_3[\mathbf{C}][\mathbf{B}]} \quad (5)$$

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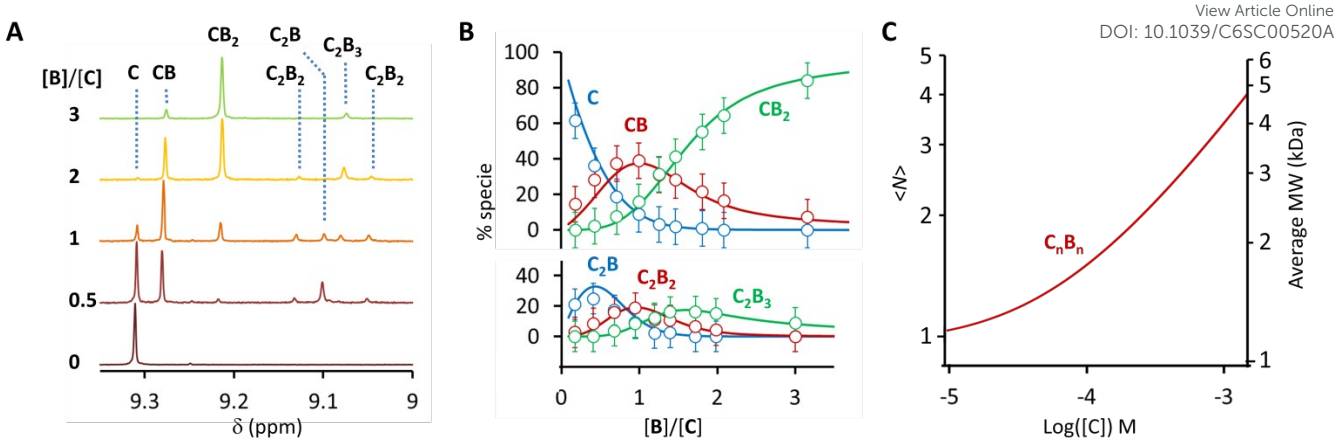

Figure 2. A. Section of the ^{1}H-NMR spectra of mixtures of **B** and **C**, showing the signal assigned to the β hydrogen of the porphyrin ring. The concentration of **C** is 100 μM in all cases. B. Speciation plot showing the amount of the various $\mathbf{C_nB_n}$ species as a function of the ratio [**B**]/[**C**] determined by integration of the ^{1}H-NMR signals (circles) and modelled using the values of K_1, K_2 and K_3 listed in Table 1 (continuous lines). The error bars are based on the intrinsic error of the NMR integral (c.a. 10%). C. Changes in the average number of **CB** repeats (i.e. ,<*N*>) and average molecular weight of an oligomer $\mathbf{C_nB_n}$ as a function of the total concentration of **C** (with [**C**]=[**B**]), estimated using the equilibria depicted in Figure 1B and the values of K_1, K_2 and K_3 listed in Table 1

Table 1. Binding parameters.

	K_1	K_2	K_3	K_o
UV	$1.1\times10^6 \pm 1.2\times10^5$	$9.7\times10^4 \pm 1.6\times10^4$	*nd*	*nd*
NMR	$1.1\times10^6 \pm 2.6\times10^4$	$1.0\times10^5 \pm 2.0\times10^4$	$2.3\times10^4 \pm 6.0\times10^3$	$8.4\times10^3 \pm 3.2\times10^3$

The units for the binding constants are M^{-1} in all cases. The quoted error is twice the standard deviation of the mean.

<*N*> increases steadily as the concentration of building blocks increases, typical of an isodesmic oligomerization (Fig. 2C). NMR data at concentrations of building blocks above 100 μM does however contradict this expectation. For example, according to our model, for concentration of **C** and **B** of 1 mM the dominant species should be a oligomer with 3-4 **CB** repeats, with a MW of just below 5000 Da (Fig. 2C). In practice, the ^{1}H-NMR signals disappear, indicating the presence of very large oligomers (i.e. polymers) (Fig. 3A). This sudden increase in the linear assembly size is attributed to a cooperative effect that is not accounted for in the modelling.

At concentrations when the ^{1}H-NMR signals start decreasing (i.e., above 100 μM) a new, red-shifted Soret band appears in the UV spectrum (Fig. 3A and 3B). Red-shifted Soret bands are indicative of the presence of *J*-type aggregates between porphyrin rings, were the rings are staked in a staggered fashion.[34,35] A possible arrangement that could lead to a *J*-type aggregate is a dimer of polymers. In the dimer, porphyrin rings intercalate in the space between the two rings of a second polymer, driven by the hydrophobic effect (Fig. 3C).

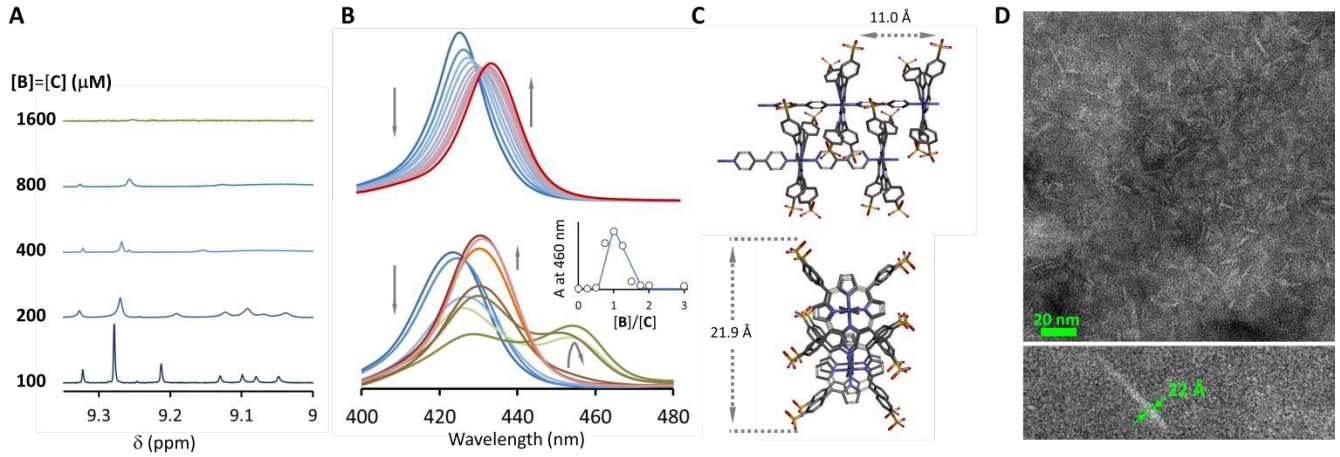

Figure 3. Evidence of polymer dimerization. A. Changes in the ^{1}H NMR signal assigned to the β proton of **C** as the concentration of building blocks increases. The buffer used is 5 mM sodium phosphate, pH 7.20. B. Changes in the Soret band of the UV spectrum of **C** as the ratio [**B**]/[**C**] changes from 0 to 3 in buffer phosphate, 5 mM, pH 7.20. In the top panel the concentration of **C** was 50 μM and in the bottom panel it was 400 μM. The inset shows the changes in absorbance at 460 nm for the lower panel experiment (empty circles) and the fit to a polymer dimerization model (blue line). C. 3D model of the dimeric form $(\mathbf{C_nB_n})_2$ of the oligomer $\mathbf{C_nB_n}$, based on the crystal structure reported in reference 34. (See Supplementary Information for details). D. Negative stain TEM image of a mixture of **C**:**B** in a ratio 1 to 1. The bottom section shows a zoom-in to an isolated single fibre together the estimated width. The average thickness of the needle-like features in 22 ± 2 A (at 95% confidence level, see Supplementary Information for details)

A number of additional experimental data support this interpretation: (i) crystal structures of **C**-**B** complexes described in the literature show the same arrangement (Fig. 3C; see also Supplementary Information and Fig. S3);[36] (ii) electron microscopy experiments, that show the presence of needle-like structures with a thickness that is consistent with the width of the dimeric oligomer (Figure 3D, and Fig. S4) and (iii) the addition of salt over mixtures of **C** and **B** at low concentrations, that triggers the rise of the red-sifted band in the UV spectrum (Fig. S5). With K_1 , K_2 and K_3 (and therefore K_o) independent of the salt concentration (see Table 1 and Table S2) this behaviour is attributed to the decrease of the electrostatic repulsion between polymers bearing multiple anionic moieties at higher ionic strength, favouring their dimerization. Changes in the ^{1}H-NMR and UV spectrum when the concentration of **C** and **B** is around 1 mM can be modelled if we take into account the dimerization of the polymers, leading to the double-stranded polymers $(\mathbf{C_nB_n})_2$, with lateral association constant K_{nl} (Fig. 4A)

$$K_{nl} = \frac{[(\mathbf{C_nB_n})_2]}{[\mathbf{C_nB_n}]^2} \qquad (6)$$

In each strand, the repeating units **CB** can be seen as a binding site, so that dimerization of the polymer results from the binding of two multivalent single-stranded polymers. K_{nl} can therefore be expressed as a function of 3 parameters: a lateral association constant per unit repeat (i.e., **CB**), K_l , the effective molarity *EM*, a parameter that gives a measure of the local concentration of complementary binding sites in a complex held by multiple interactions, and the number of polymer repeats *n* in each of the strands as follows:

$$K_{nl} = EM^{n-1}K_l^{\,n} \quad (7)$$

For the oligomerization of **C** and **B**, the formation of three types of oligomers is taken into account (**B** capped, **C** capped and with mixed capping, see Fig. 1B). For the dimerization of the polymer we make two assumptions that considerably simplify the system (Fig. 4A): (i) only mixed capping polymers exist (i.e., with equal **C** and **B** units). This assumption supported by the fact that, for polymers, the average ratio **C**/**B** is equal to 1. (ii) The initial concentration of complex **CB**, $[\mathbf{CB}]_0$, is dictated solely by K_1. This assumption is supported by the fact that K_1 is much larger than all the other association constants (Table 1). Therefore we view the polymerization process as the assembly of equal **CB** monomers. We call the concentration of **B** and **C** that are not involved in the formation of **CB** $[\mathbf{B}]_x$ and $[\mathbf{C}]_x$ respectively. The polymer can be disassembled by the addition of excess **B**, that is, increasing $[\mathbf{B}]_x$ (leading to $\mathbf{CB_2}$) or **C** that is, increasing $[\mathbf{C}]_x$ (leading to $\mathbf{C_2B}$), as dictated by K_2 and K_3 respectively (Fig. 4A). The equation that relates all the concentrations is (see Supplementary information for detailed derivation):

$$[\mathbf{CB}]_0 = \frac{K_2[\mathbf{CB}][\mathbf{B}]_x}{(1+K_2[\mathbf{CB}])} + \frac{K_3[\mathbf{CB}][\mathbf{C}]_x}{(1+K_3[\mathbf{CB}])} + \frac{[\mathbf{CB}]}{(1-K_o[\mathbf{CB}])^2} + \frac{2K_l[\mathbf{CB}]^2}{(1-K_lEMK_o^2[\mathbf{CB}]^2)^2} \qquad (8)$$

where the first two members are the concentrations in equilibrium of $\mathbf{CB_2}$ and $\mathbf{C_2B}$ respectively, the third member the concentration of **CB** repeating units within single stranded oligomer of any length and the fourth member is the concentration of **CB** units within double stranded polymers of any length. Equation (8), combined with the mass balance (equations (S45) and (S46)), allows calculating the concentration of free **CB** and therefore of all the species, including that of polymer dimer $(\mathbf{C_nB_n})_2$, at any initial concentration of building blocks (Figure S6). Equation (8) also shows that for the polymer dimer to form to any meaningful extent, (i.e., for multivalence cooperativity to be positive) the product K_lEM must be larger than 1.[2] Around 460 nm the absorbance *A* in the UV spectrum is solely due to the red-shifted band assigned to the polymer dimer, $(\mathbf{C_nB_n})_2$, and can be written as (see Supplementary Information for details):

$$A = \varepsilon \frac{2K_l[\mathbf{CB}]^2}{(1-K_lEMK_o^2[\mathbf{CB}]^2)^2} \qquad (9)$$

where ε is the molar extinction coefficient per **CB** unit at the wavelength under study (Fig. 3B). With all the constants independently determined, except for K_l and *EM*, the model fits remarkably well to the experimental changes in absorbance at 460 nm (Fig. 3B), further supporting the polymer-dimer model. Using a value of *EM* of 1 M, (a reasonable value in supramolecular systems[37]) the fitting produced a value of K_l of 6.1 M^{-1} in sodium phosphate 5 mM, pH 7.20. The constant is small, but due to multivalence cooperativity, polymers dimerize quantitatively. As a consequence, there is a sudden increase in the population of large double-stranded polymers when a critical concentration is reached (Fig. 4B). The average number of repeat units in the double-stranded polymer, $<N_2>$ can be calculated from the concentration of complex **CB** as follows:

$$< N_2 >= \frac{1}{1-K_o{}^2EMK_l[\mathbf{CB}]^2} \qquad (10)$$

Changes in polymer length (and therefore molecular weight) predicted by equation (10) are consistent with changes in the ^{1}H NMR spectrum, where the rapid rise of the polymer average molecular weight and the increase of overall large polymer population leads to the line broadening observed (Fig. 3A and Fig. 4).

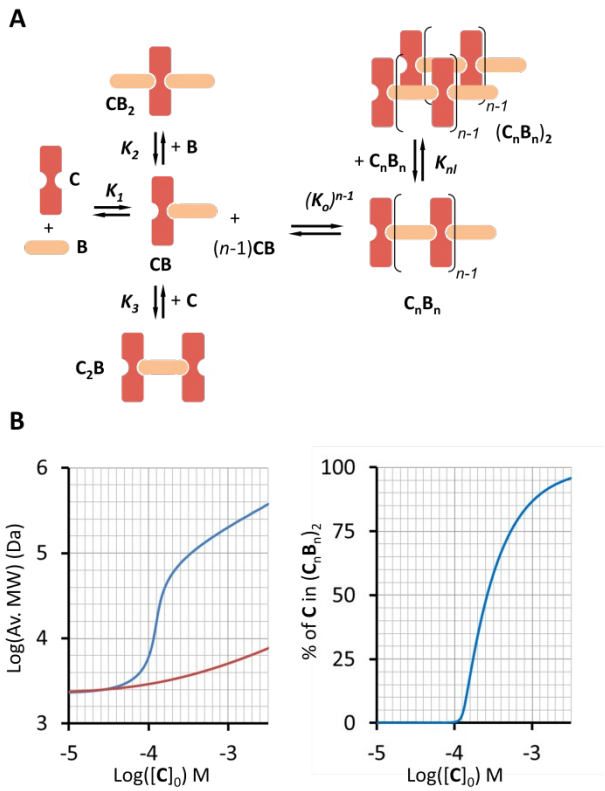

Figure 4. A. Equilibria that describe the oligomer dimerization model. B. Left panel: Changes in the average MW of the oligomer dimer $(\mathbf{C}_n\mathbf{B}_n)_2$ (blue trace) as the total concentration of **C** increases ([**B**] = [**C**]). For comparison, the increase in average oligomer molecular weight in the absence of dimerization is also shown (red trace). The right panel shows the change in percentage of **C** in $(\mathbf{C}_n\mathbf{B}_n)_2$ as a function of the total concentration of **C**

Our model, which accounts for the formation of a double-stranded polymer, can be expanded to the formation of multi-stranded polymers with *m* strands. Unlike the number of polymer repeats *n*, which can be a very large, *m* is a discrete number. For simplicity and general applicability, we consider that polymers are formed by the self-assembly of only one type of building block, that we call **M**, rather than the two components **C** and **B**. Monomer **M** can assemble in the longitudinal direction, forming single stranded polymers of *n* units $\mathbf{M}_n$ (Fig. 5A, equilibrium 1). The assembly is isodesmic, with oligomerization constant K_o. The single strand $\mathbf{M}_n$ can interact laterally with up to another *m*-1 strands to form a multi-stranded polymer of *m* strands $(\mathbf{M}_n)_m$. The lateral assembly is also isodesmic, with lateral association constant $K_{nl} = K_l^n\, EM^{n-1}$, according to equation (5) (Fig. 5A, equilibrium 4). We can depict an alternative mechanism of formation of the polymer with *m* strands $(\mathbf{M}_n)_m$, with **M** first assembling laterally with up to *m*-1 molecules of **M** to produce the complex $\mathbf{M}_m$, following an isodesmic mechanism with an stepwise lateral assembly constant K_l (Fig. 5A, equilibrium 2). The complex $\mathbf{M}_m$ then assembles linearly with another *n*-1 $\mathbf{M}_m$ complexes, following an isodesmic mechanism with stepwise oligomerization constant K_{mo}, which is related to K_o as follows

$$K_{mo} = K_o{}^m EM^{m-1} \quad (11)$$

In other words, **M** can assemble in two orthogonal directions, but the lateral assembly ends up at a relatively small number *m* while the longitudinal assembly is open ended (Fig. 5A). Irrespectively of the route of formation of the multi-stranded polymer, the expression that relates $[\mathbf{M}]_0$ and [**M**] for a polymer composed of a bundle of *m* strands can be written as:

$$[\mathbf{M}]_o = \sum_{i=1}^{i=m} \frac{iK_l{}^{i-1}[\mathbf{M}]^i}{(1-K_o^i EM^{i-1}K_l{}^{i-1}[\mathbf{M}]^i)^2} \quad (12)$$

and the average number of $\mathbf{M}_i$ unit repeats (with *i* values from 1 to *m*) in a multi-stranded polymer with *i* strands, $\langle N_i \rangle$,is (see Supplementary Information for detailed derivation of the equations):

$$\langle N_i \rangle = \frac{1}{1-K_o{}^i EM^{i-1}K_l{}^{i-1}[\mathbf{M}]^i} \quad (13)$$

Using equations (12) and (13) and knowing K_o , *EM* and K_l it is possible to determine the changes in concentration for polymer bundles of any number of strands, and average number of repeats in the polymer, as the total concentration of the monomer changes. These simulations reveal the following features of the system (Fig. 5): (i) the only species present to any meaningful extent are the monomer, short, single stranded ,oligomers and the multi-stranded polymer with maximum number of strands *m* while polymers with an *i* number of strands less than *m* form only to a very limited extent (Fig. 5B) (ii) the multi-stranded polymer forms following a typical nucleation-growth mechanism, appearing suddenly when a nucleation concentration is reached, with the polymer growing rapidly around this concentration (Fig. 5B and C); (iii) the preferred length of the polymer depends on the maximum number of strands *m* (Fig. 5C); and (iv) the onset of formation of the multi-stranded polymer is very sensitive to small variations on K_l or K_o (Fig. 5D).

The very high sensitivity of the multi-stranded polymers formation to subtle changes in either the pair-wise binding constants or the concentration of building block **M** is attributed to the fact that the system switches from the assembly of the monomer (equilibria 1 and 2, Fig. 5A) to the assembly of the nucleus, which is multivalent (equilibria 3 and 4, Fig. 5A) when a critical concentration of **M** is reached. However, it is not the multivalence, on its own, that provides the on-off assembly behaviour, but rather the fact that the nucleus is multivalent relative to the monomer. For example, a multivalent monomer **M'**, analogous to the nucleus $\mathbf{M}_m$, will yield polymers to the same extent as $\mathbf{M}_m$ (and to a much larger extent than **M**) but the assembly process will be isodesmic, showing a smooth dependence of assembly with the concentration of **M'** (equilibrium 0, Fig. 5A and 5E). By contrast, for **M**, the assembly is dictated by the properties of $\mathbf{M}_m$ only above a certain critical concentration. At this concentration, the length and stability of the polymer formed from the assembly of **M** building blocks becomes comparable to that formed from **M'**, giving rise to an sudden increase in the concentration of the assembled species (Fig. 5A and 5E.)

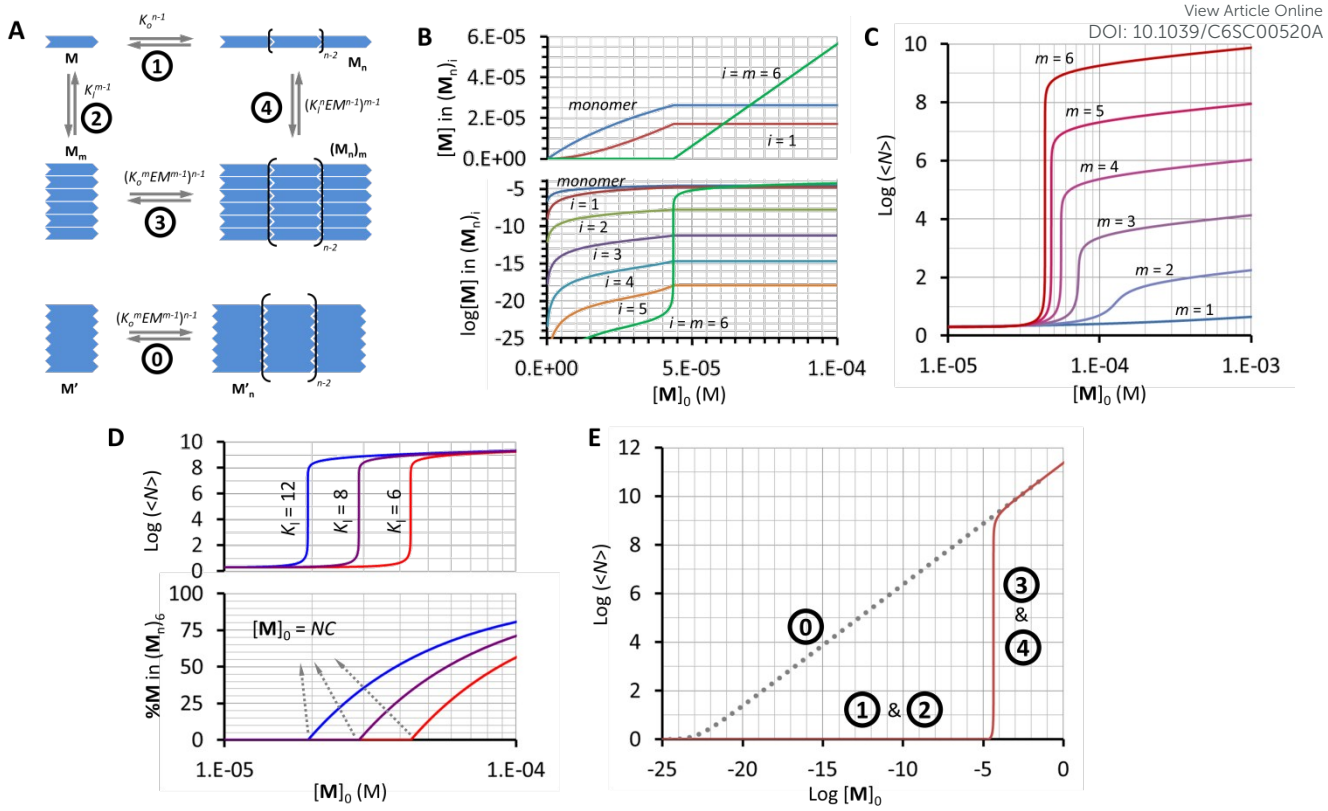

Figure 5. Multi-stranded polymers. A. Thermodynamic cycle for the formation of an multi-stranded polymer from **M** (1, 2, 3 and 4, top scheme) and equilibrum of oligomerization of **M'** (0, bottom scheme). B. Changes in the concentration of building block **M** in the species of the form $(\mathbf{M}_n)_i$ as function of the total concentration of **M**. A logarithmic scale in the y-axis is needed to show the changes in concentration of multi-stranded polymers with $i < m$ (bottom panel) . C. Average number of repeats <*N*> in multi-stranded polymer with different number of maximum number of strands *m* as a function of the total monomer concentration, calculated using the multi-stranded polymer model D. Average number of repeats <*N*> (top) and percentage of monomer (bottom) in an hexa-stranded polymer (m=6) for different values of K_i and as a function of the total concentration of monomer . E. Changes in the average length of an hexa-stranded polymer as a function of the total concentration of **M'** (dotted line) and **M** (continuous line), The number labels show the dominating equilibrium according to panel A. The parameters used to generate these plots are K_o = 8400 M^{-1} , K_o' = $(8400)^6$ M^{-1} , K_i = 6 M^{-1} and EM = 1 M, except when stated in panel D.

The switching point in assembly regime occurs at the nucleation concentration, *NC*. The *NC*, can be written as a function of the constants K_i and K_o, and the maximum number of strands *m*, as follows (see Supplementary Information for detailed derivation of the equation):

$$NC = \frac{(K_i EM)^{1/m}}{K_i EM K_o (1-\frac{(K_i EM)^{1/m}}{K_i EM})^2} \qquad (14)$$

Above the *NC*, the size of the oligomer can be expressed as a function of K_o, K_i , *EM* and the number of strands *m* as follows:

$$log < N_m >= 0.5 m log K_o + 0.5(m-1) log EM - 0.5 log m + 0.5 log([\mathbf{M}]_o - NC) \qquad (15)$$

A conveniently simple expression can be obtained by combining equation (14) and (15) for a total concentration of the monomer that is twice that of the *NC* (see Supplementary Information for detailed derivation of the equations):

$$log < N_m >= 0.5(m-1)\log K_o EM - 0.5 log m - 0.5\left(\frac{m-1}{m}\right)\log K_i EM - log(1-(K_i EM)^{(1-m)/m}) \qquad (16)$$

Equation (16) allows calculating the expected size of a multi-stranded polymer as a function of parameters that are characteristic of the monomer, that is, the association constants, the effective molarity and the number of strands in the polymer.

Conclusions

We have shown that the sequential assembly of cobalt metalloporphyrin **C** and 4,4'-bipyridine **B** leads to double stranded polymers, following a nucleation growth mechanism. The mathematical model derived from the analysis of the spectroscopic data shows that the nucleation-growth mechanism is consequence of a multivalence cooperative effect.

The model was applied to the assembly of multi-stranded polymers with any number of strands. Simulations show that this model accounts well for the all-or-nothing assembly typical of a nucleation-growth mechanism. The overall process can be summarized as a thermodynamic cycle. In the first part of the cycle, monomers assemble into nuclei, following an isodesmic mechanism. In the second part, the nuclei assemble

into polymers, also following an isodesmic mechanism. The cooperative behaviour arises from the fact that the assembly of the nuclei is much more efficient than that of the monomers, on account of the multivalent effect. This model offers a simple interpretation of the all-or-nothing assembly observed in many natural (such as microtubules) and artificial (such as small molecule based gelators) polymers. For example, recent studies have found that the assembly of microtubules is better explained as a combination of the lateral and longitudinal binding energies of tubulin, consistent with our model, rather than the classical helicoidal assembly model.[38] Moreover, the de-assembly or "catastrophe" following small conformational changes of the tubulin building blocks is also consistent with the all-or-nothing assembly following small changes in the individual pairwise binding constants,[6] as predicted by our model. Also consistent with our model, it has been observed that the degree of cooperativity in the assembly of some small molecule based gelators depends on the thickness of gel fibres, which in turns depends on the number of strands that form the fibre.[20].

Our model does not exclude the possibility that other cooperative effects of allosteric origin may also play a role in supramolecular polymerization, especially for single-stranded polymers. However, this work shows that for multi-stranded polymers, multivalence cooperativity plays a central role that our model allows to describe quantitatively. For these polymers, the maximum number of strands *m*, together with the lateral and oligomerization constants are the key parameters that dictate the preferred size of the oligomer and the nucleation concentration. Since these parameters depend ultimately on the structure of the building blocks, our model is also a useful tool to predict the assembly properties of de-novo synthesized molecules. We believe however that our interpretation of the all-or-nothing assembly of supramolecular polymers, whereby the chelate effect is triggered after a multivalent nucleus if formed, can be applied to the formation of any kind of long range assembly that follows a cooperative, all-or-nothing behaviour. Our model can therefore be expanded to include processes as diverse as crystallization of small molecules or the formation of cell membrane adhesion points. Regarding the latter, previous work from our group has led to an analogous assembly model for membrane embedded receptors .[39,40] Current work in our laboratories is directed at demonstrating the general applicability of the model and the specific applicability to cell-cell communication processes involving cell membrane contacts and in the design of multi-stranded supramolecular polymers with predictable assembly properties.

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Notes and references

1 A. Whitty, *Nat. Chem. Biol*., 2008, **4**, 435.
2 C. A. Hunter, H. L. Anderson, *Angew. Chem. Int. Ed*., 2009, **48**, 7488.
3 M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem. Int. Ed.,* 1998, **37**, 2754.
4 J. D. Badjic, A. Nelson, S. J. Cantrill, W. B. Turnbull, J. F. Stoddart, *Acc. Chem. Res*. 2005, **38**, 723.
5 A. Mulder, J. Huskens, D. N. Reinhoudt, *Org. Biomol. Chem.,* 2004, **2**, 3409.
6 D. A. Fetcher, R. D. Mullinds, *Nature*, 2010, **463**, 285.
7 T. F. A. De Greef, M. M. J. Smulders, M. Wolffs, A. P. H. J. Schenning, R. P. Sijbesma, E. W. Meijer, *Chem. Rev*., 2009, **109**, 5687.
8 T. Aida, S. I. Stupp, E. W. Meijer, *Science* 2012, **335**, 813.
9 A. P. H. J. Schenning, E. W. Meijer, *Chem. Commun*., 2005, 3245.
10 F. J. M. Hoeben, A. P. H. J. Schenning, E. W. Meijer, *ChemPhysChem*, 2005, **6**, 2337.
11 R. Abbel, C. Grenier, M. J. Pouderoijen, J. W. Stouwdam, P. E. L. G. Leclere, R. P. Sijbesma, E. W. Meijer, A. P. H. J. Schenning, *J. Am. Chem. Soc.*, 2009, **131**, 833.
12 L. Milanesi, C. A. Hunter, N. Tzokova, J. P. Waltho, S. Tomas, *Chem. Eur. J.*, 2011, **17**, 9753.
13 E. J. Howe, B. O. Okesola, D. K. Smith, *Chem. Commun.*, 2015, 7451.
14 A. R. Hirst, B. Escuder, J. F. Miravet, D. K. Smith, *Angew. Chem. Int. Ed.*, 2008, **47**, 8002.
15 A. S. Weingarten, R. V. Kazantsev, L. C. Palmer, M. McClendon, A. R. Koltonow, A. P. S. Samuel, D. J. Kiebala, M. R. Wasielewski, S. I. Stupp, *Nature Chem.*, 2014, **6**, 964.
16 R. N. Shah, N. A. Shah, M. M. D. Lim, C. Hsieh, G. Nuber, S. I. Stupp, *Proc. Nat. Acad. Sci. USA,* 2010, **107**, 3293.
17 G. A. Silva, C. Czeislszer, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler, S. I. Stupp, *Science,* 2004, **303**, 1352.
18 J. Boekhoven, S. I. Stupp, *Adv. Mater.* 2014, **26**, 1642.
19 D. H. Zhao, J. S. Moore, *Org. Biol. Chem.*, 2003, **1**, 3471.
20 A. R. Hirst, I. A. Coates, T. R. Boucheteau, J. F. Miravet, B. Escuder, V. Castelletto, I. W. Hamley, D. K. Smith, *J. Am. Chem. Soc.*, 2008, **130**, 9113.
21 R. Krishnan, S. L. Lindquist, *Nature*, 2005, **435**, 765
22 S. L. Bernstein, N. F. Dupuis, N. D. Lazo, T. Wyttenbach, M. M. Condron, G. Bitan, D. B. Teplow, J.-E. Shea, B. T. Ruotolo, C. V. Robinson, M. T. Bowers, *Nature Chem.*, 2009, **1**, 326.
23 P. A. Korevaar, S. J. George, A. J. Markvoort, M. M. J. Smulders, P. A. J. Hilbers, A. P. H. J. Schenning, T. F. A. De Greef, E. W. Meijer, *Nature*, 2012, **481**, 492.
24 P. Jonkheijm, P. van der Schoot, A. P. H. J. Schenning, E. W. Meijer, *Science*, 2006, **313**, 80.
25 F. Oosawa, M. Kasai, *J. Mol. Biol.*, 1962, **4**, 10.
26 E. D. Korn, *Physiol. Rev.*, 1982, **62**, 672.
27 H. L. Anderson, *Chem. Commun.*, 1999, 2323.
28 Y. Tian, C. M. Beavers, T. Busani, K. E. Martin, J. L. Jacobsen, B. Q. Mercado, B. S. Swartzentruber, F. van Swol, C. F. Medforth, J. A. Shelnutt, *Nanoscale* 2012, **4**, 1695.
29 M. Gilbert, B. Albinsson, *Chem. Soc. Rev.*, 2015, **44**, 845.
30 C. A. Hunter, S. Tomas, *J. Am. Chem. Soc.*, 2006, **128**, 8975.
31 A. Camara-Campos, C. A. Hunter, S. Tomas, *Proc. Natl. Acad. Sci. USA,* 2006, **103**, 3034.
32 M. Bhatti, T. D. McHugh, L. Milanesi, S. Tomas, S. *Chem. Commun.*, 2014, 7649.

View Article Online
DOI: 10.1039/C6SC00520A

33 A. Ortega, D. Amoros, J. Garcia de la Torre, *Biophys. J.*, 2011, **101**, 892.
34 O. Ono, Y. Kaizu, H. Kobayashi, *J. Chem. Phys.*, 1993, **99**, 4128.
35 Y. Gao, X. Zhang,C. Ma, X. Li, J. Jiang, *J. Am. Chem. Soc.*, 2008, **130**, 17044.
36 A. Fidalgo-Marijuan, G. Barandika, B. Baza, M.-K. Urtiaga, M. I. Arriortua, *CrystEngComm*, 2013, **15**, 4181.
37 M. C. Misuraca, T. Grecu, Z. Freixa, V. Garavini, C. A. Hunter, P. W. van Leeuwen, M. D. Segarra-Maset, S. M. Turega, *J. Org. Chem.*, 2011, **76**, 2723.
38 M. K. Gardner, B. D. Charlebois, I. M. Jánosi, J. Howard, A. J. Hunt, D. J. Odde, *Cell*, 2011, **146**, 582.
39 S. Tomas, L. Milanesi, *Nature Chem.* 2010, **2**, 1077.
40 A. Grochmal, E. Ferrero, L. Milanesi, S. Tomas, *J. Am. Chem. Soc.*, 2013, **135**, 10172.