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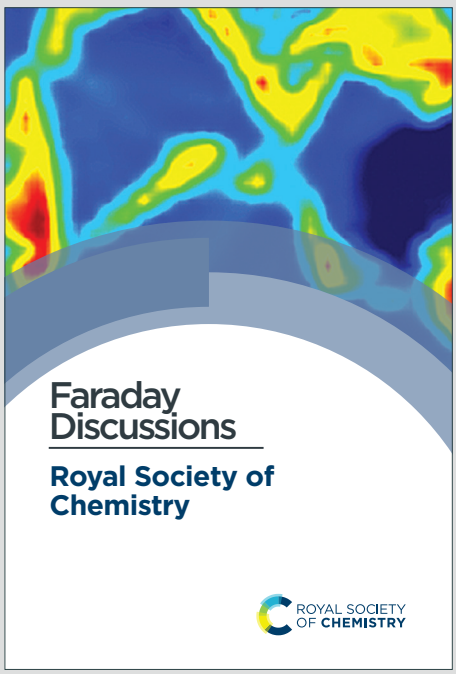

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View Article Online
DOI: 10.1039/D5FD00016E

ARTICLE

Autoinduction through the coupling of nucleation-dependent self-assembly of a supramolecular gelator and a reaction network.

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Jamie S. Foster[a] and Gareth O. Lloyd*[b]

Autocatalytic and/or self-replicating systems are important aspects of understanding the link between living systems (origins of life) and chemical networks. As a result, many scientists around the world are attempting to better understand these phenomena by producing chemical networks and linking them to self-assembly and pathway complexity (systems chemistry). We present here a superficially autocatalytic, self-replicating system by utilising dynamic imine chemistry coupled with self-assembling supramolecular hydrogelation kinetics that is driven by a nucleation autocatalytic cycle (autoinduction). The dynamic nature of the imine bond within water allows "error checking" correction and driving of the imine equilibrium to starting materials but when coupled to the self-assembly gives rise to one reaction product from a possible thirteen intermediates and/or products (of a mixed four-step reaction). This product represents a thermodynamic minimum within the system's and reaction network's energy landscape. The self-assembly in solution of the replicator results in the formation of supramolecular polymers which would normally markedly reduce the catalytic efficiency of the system if a template mechanism of autocatalysis is in play. By overcoming the limiting effects of the self-assembly process it is possible to demonstrate exponential growth in replicator concentration once nucleation has occurred. It is only once the completed imine can undergo non-reversible tautomerisation that the product is prevented from reacting with water. We thus suggest that this sigmoidal kinetic characterisation is not inherent to autocatalysis kinetics (lowering reaction barriers and/or templating) but rather a result of the nucleation-based assembly allowing for intermediates to be prevented from reacting with water in a water-deficient environment (an autoinduction autocatalytic mechanism). Not only does this study provide a basis with which to explore aspects of self-replication connected with self-assembly, but also explores how nucleation and self-assembly growth can play a crucial role in self-replication. By controlling the kinetics of the autocatalytic chemical reaction at one end of the hierarchical assembly process we can influence the physical properties of the supramolecular gel at the other. This may have wide-ranging applications with in situ formed small molecular gelators where specific mechanical properties (rheology) are desired.

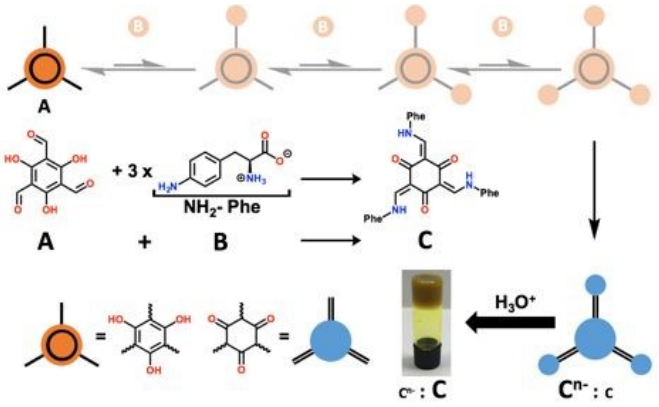
Introduction

Self-replication, or autocatalysis, relating to autoinduction is a keystone of chemical biology and is an important concept in the understanding of chemical complexity.[1–3] Supramolecular chemistry has long been recognised as a key part of the process involved in an autocatalytic chemical reaction. The well-recognised design principle of template-directed ligation of reactant components of the replicator/product describes this supramolecular recognition function.[1–3] However, subsequent dissociation of this duplex formed after linkage of the building blocks between "two" products is necessary if exponential growth of the replicator is to be seen.[4] This exponential growth results from the liberation of two replicators from the duplex that can each mediate another round of replication. When coupled to a dynamic bond formation, replication can inhibit the thermodynamic equilibration of the system.[5] With this in mind, designing biomimetic gelatinous materials such as hydrogels has necessarily included aspects of systems chemistry.[6–9] This has been due to nature's exquisite ability to control assembly and disassembly, often resulting in non-equilibrium states.[10–12] This control by nature often arises from catalysed chemical processes, most of which show autocatalytic behaviour. The most successful biomimicry in gelation by small molecule gels to date have included dissipating materials, chemical Darwinian-selection, and catalysed formation.[13] There has also been a keen increase in interest on the self-assembly of peptides (amyloid fibrils in particularly) which show autocatalytic assembly and there has been a recognised association of the nucleation/growth kinetics to these processes.[14] This, connected with classical nucleation theory, shows that crystal growth or nucleation determine self-assembly are naturally autocatalytic in terms of their kinetics.[15] However, there are relatively few autocatalytic gelation[16] or supramolecular polymerisation processes described so far with simple building blocks.[17] Therefore we aimed to produce and study an autocatalytic reaction involved in a gel forming process that may be valuable in studies of biomimetic gelation and their subsequent materials by coupling a reaction network with an autocatalytic nucleation-based self-assembly (Scheme 1).[18,19] We have used a combination of the core

[a] Institute of Chemical Sciences, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, Scotland, United Kingdom, EH14 4AS.
[b] Department of Chemistry, School of Natural Sciences, Joseph Banks Laboratories, Lincoln, LN6 7DL, United Kingdom.

† Footnotes relating to the title and/or authors should appear here.
Supplementary Information available: . See DOI: 10.1039/x0xx00000x

A (1,3,5-triformylglucinol) and periphery **B** (4-amino-*L*-phenylalanine) that react together through the imine dynamic covalent chemistry resulting in the potential reaction network. The reaction network however only appears to form one observable product and no other potential predict nor intermediate is observed, unusually. By developing an understanding of these reaction kinetics for the formation of the individual supramolecular building blocks we hoped to be able to control the physical properties, such as mechanical strength, of any resulting gel.[20] Control would arise from the trapping a specific concentration of the gelator through a mass transfer limitation mechanism (Le Chatelier's principle), stopping the reaction reaching completion.[21] A necessitated discussion based on what gives rise to these perceived autocatalytic kinetics when we connect our reaction networks and self-assembly will also be presented, and again there appears to be a connectivity between nucleation/growth self –assembly, Le Chatelier's principle, and equilibria of the reactions.



Scheme 1. Simplified linear reaction scheme for the reaction, from combination of the starting materials, **A** (1,3,5-triformylglucinol) and **B** (4-amino-*L*-phenylalanine). Mixing of **A** and **B** occurs in water at pH 8 and results in formation of the deprotonated non gelating but supramolecular fibre assembling species $\mathbf{C}^{n-}$. Acidification of $\mathbf{C}^{n-}$ results in protonation giving rise to the formation of the LMWG **C**.

Results and Discussion

The design of our self-replicating system is based on the initial formation of imine bonds. There are two processes on which the system is reliant. The classic imine dynamic covalent chemistry involving the aldehyde and amine groups.[22] This is followed by a enol-keto tautomerisation (Scheme 1, Fig. S1). Mixing **A** and **B** in water at pH 9 gives only one isolatable product, $\mathbf{C}^{n-}$. We propose the dynamic nature of the imine bond results in a system where the mono-, di- and tri- imine species are not observed due to their lack of thermodynamic stability. However, reaction to form the tri-reacted imine may induce an enol-imine to keto-enamine tautomerization.[23] This traps the tri-reacted species in the keto tautomeric form, $\mathbf{C}^{n-}$.[24] A species which represents a thermodynamic minima for the reaction between **A** and **B** (Scheme 1). The amino acid **B** features two amine groups, both have the potential to react with the aldehyde groups of **A**. However, spectroscopic evidence (see ESI, Figure S9-10) shows only one product resulting from reaction at the aromatic amine of **B** to give $\mathbf{C}^{n-}$. And we believe the alternative products where the alkyl amine has reacted with an aldehyde would result in a highly soluble compound that cannot self-assemble at this pH in water driving its equilibrium formation back to starting material in these conditions.

The formation of $\mathbf{C}^{n-}$ could be monitored by HPLC and ^{1}H NMR (sections 9, 14, 15, 18 ESI). This was done in water with standard experimental conditions of pH 9 at 20 °C. The HPLC analysis measured absorption at 290 nm at retention times corresponding to **A** and $\mathbf{C}^{n-}$. ^{1}H NMR analysis focused on the conversion of the aldehyde peak of **A** at 9.6 ppm to the enamine peak of $\mathbf{C}^{n-}$ at 7.5 ppm.

Two solutions containing **A** (28 mM) and an excess of **B** take 72 hours after mixing to completely react to form $\mathbf{C}^{n-}$. A plot of [$\mathbf{C}^{n-}$] against time gives rise to a sigmodal profile that suggests an autocatalytic process (Fig. S13 and S16). A subsequent plot of the calculated autocatalysis on templating can reduce catalyst efficiency as replicator molecules may form dimers or higher ordered structures. This results in the system showing a non-exponential increase in product concentration, $0.5 \leq p < 1$, such a system's growth is said to be parabolic. To determine the order of the reaction, in terms of product concentration, the reaction was seeded with varying concentrations of previously isolated $\mathbf{C}^{n-}$ and the initial rate of $\mathbf{C}^{n-}$ formation was calculated (Fig. S14, S17 and S20). Kiedrowski showed that by plotting log d[$\mathbf{C}^{n-}$]/dt against log[$\mathbf{C}^{n-}$], where [$\mathbf{C}^{n-}$] refers to the concentration of the seed added, p can be determined from the gradient of the line. We therefore determined the relationship, reaction rate for $\mathbf{C}^{n-}$ formation (d[C]/dt) against time results in a bell shaped profile, again indicative of autocatalysis.

$$\frac{d[\boldsymbol{C^{n-}}]}{dt} = k[\boldsymbol{C^{n-}}]^p + x$$

Equation (1), above, is the general rate equation for an autocatalytic process.[25] k[$\mathbf{C}^{n-}$]^p is the autocatalytic term, x is the non-autocatalysed reaction and p is the order of reaction with respect to the product. When p = 1 an autocatalytic system will exhibit exponential increase in product concentration. The reliance of rate = 2.3[$\mathbf{C}^{n-}$]^1 (Figure 1). We can see p = 1, thus showing seeding resulted in an autocatalytic system with an exponential increase in product/replicator occurring.

The face-to-face, π-π stacking of $\mathbf{C}^{n-}$ molecules, evident in the powder X-ray diffraction patterns of dried samples (Fig. S34-35)), is proposed as the supramolecular interaction in the product template-based self-assembly and must be evident during an mechanism for the autocatalytic kinetics.[26] The efficiency of the catalytic process relies on the number of accessible faces of $\mathbf{C}^{n-}$ molecules in solution. As the reaction progresses it is possible to measure an increase in reaction solution viscosity, an indication of the supramolecular polymerisation of $\mathbf{C}^{n-}$. Utilising isolated **C** and dissolving it in pH 9 water, an Increasing concentration of $\mathbf{C}^{n-}$ in solution also results in an increase in viscosity. The viscosity of the solution shows a dramatic reduction at temperatures > 30 °C. This results from dissociation of the π-π stacking interactions between monomers of $\mathbf{C}^{n-}$ that have formed supramolecular polymers.

There are two methods by which propagation of the supramolecular polymers can occur during the reaction. The conglomeration of individual $\mathbf{C}^{n-}$ molecules in solution that form through the non-catalysed process and/or inclusion of an intermediate of the reaction network into the polymer chains potentially catalysing the formation of $\mathbf{C}^{n-}$.

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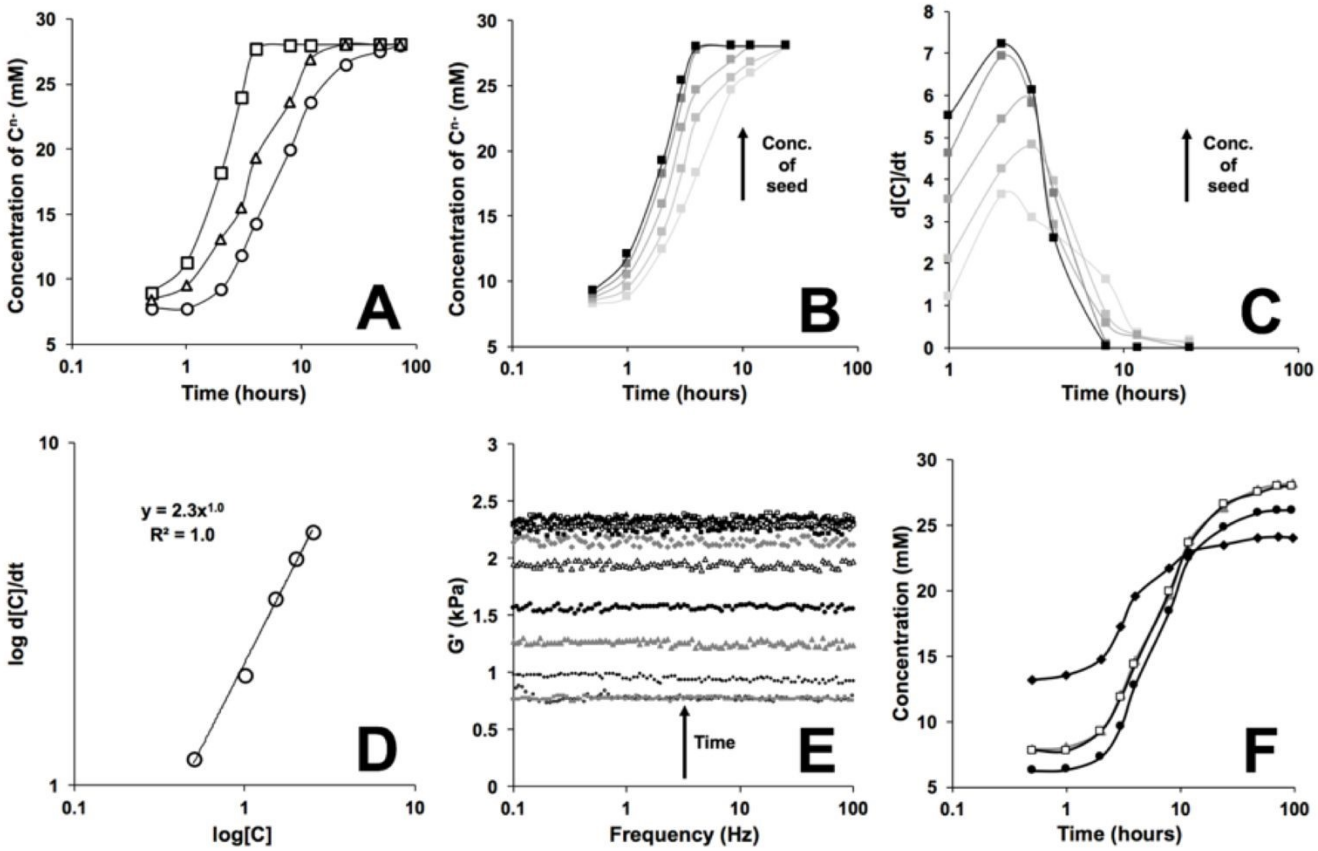

Figure 1. Data for the autocatalytic reaction to produce $\mathbf{C}^{n-}$ and the mechanical properties arising from the hydrogel. Graph A [$\mathbf{C}^{n-}$] in over time. Standard conditions (○), stirring at 1000 rpm (△), addition of previously isolated **C** seed at a concentration of 2.5 mM (□). Graph B [$\mathbf{C}^{n-}$] increase over time with increasing [seed]. Graph C rate of formation of $\mathbf{C}^{n-}$ over time with increasing concentration of seeding $\mathbf{C}^{n-}$. Graph D initial rate of formation of $\mathbf{C}^{n-}$ plotted against concentration of seeding $\mathbf{C}^{n-}$. Graph E frequency sweep experiments for gels set at different times after the initial mixing of **A** and **B** and the subsequent acidification to form **C** (coloured version, Figure S12, with clearer legends available). 0.5 h ○, 1 h ■, 2 h ◆, 3 h ▲, 4 h ●, 8 h △, 12 h ◆, 24 h ■, 48 h ◇, 72 h □, 96 h ◆. Graph F [$\mathbf{C}^{n-}$] over time determined by UV-Vis ●, HPLC □, ^{1}H NMR spectroscopy ▲, rheological using the cellular solid model ◆.

The $\mathbf{C}^{n-}$ molecules that form through the reaction process connected to being part of the supramolecular polymers may fail to dissociate from the polymer, resulting in an increase in polymer length.The presence of supramolecular polymers results in the effectiveness of the autocatalytic process observed experimentally being much lower than the theoretical maximum, as each static linear polymer chain only has two catalytically active end molecules/sites. It is well recognised now that this addition catalytically (similar to the template process of self-replication) would not lead to the kinetics observed. In our reactions the replicator:product "duplexes" must be dynamic and related to the supramolecular polymerisation as described by Otto and co-workers in their description of the of fibre elongation/breakage mechanism and many others who have observed autocatalytic kinetics during self-assembly of supramolecular polymers/fibrils.[27] In an attempt to mitigate the effects of the supramolecular polymer formation on the perceived catalytic activity the solution was subjected to physical agitation (stirring at 1000 rpm with a magnetic stirrer, see ESI sections 12 and 13 for details).[26] Stirring of the solution was started immediately on mixing **A** and **B** and continued until the maximum concentration of $\mathbf{C}^{n-}$ was reached. With stirring, the reaction completed within 24 hours, much quicker than the same reaction without stirring. This confirms the autocatalytic kinetics is related to some form of assembly process, as stirring breaks/sheers the polymer chains into smaller units, increasing the number of accessible sites for chemical assembly or reactivity (we cannot determine which). With these observations, data, chemical reactivity, self-assembly, and unknowns in hand, we are naturally led to a discussion (hyperbole) of how do we describe this connectivity between the reaction network and self-assembly arising in an autoinduction observation.[1]

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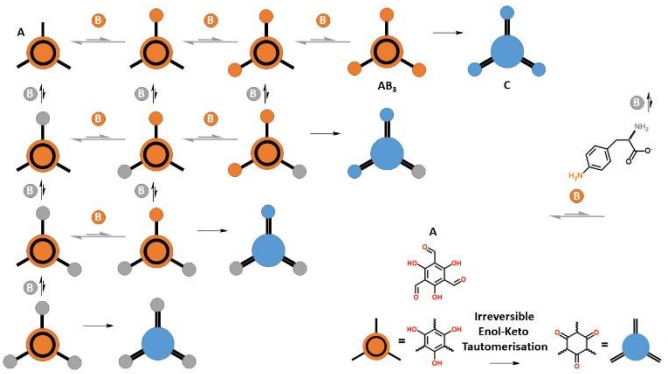

Figure 2. Reaction Network showing the thirteen possible products from the mixing of **A** (1,3,5-triformylglucinol) and **B** (4-amino-*L*-phenylalanine) in water at a pH of approximately 8. **A** can react with **B** through the aniline functional group (horizontal equilibrium arrows (orange)) or the alkyl amine functional group (vertical equilibrium arrows (grey)). Both equilibria are biased towards starting materials due to water. The irreversible enol-keto tautomerisation only occurs once all three aldehyde functional groups have reacted, shown as a horizontal reaction unidirectional arrow. Only the produce **C** is analytically observed. It results from the tautomerisation of **AB_3** (the thrice reacted aniline product).

We can divide this discussion into two: (1) Are any of the reaction steps (Figure 2) catalytically enhanced during the process resulting in a self-replication mechanism; (2) Is the self-assembly autocatalytic in nature (Figure 3) and is there a connectivity between the reaction network and this autocatalytic assembly cycle?

The first reflexion of the reaction to take note of is that there is no analytical observation of any of the intermediates of the reaction network. This is a classic analytical limitation of many chemical reactions (most reactions have time scales/kinetics far quicker than most analytical tools) but unusual given the network possibilities (13 intermediates and/or products plus an addition unlikely five products associated with the tautomerisation of the five intermediates with free aldehyde groups). An additional fact is the reaction takes three days to complete. We and many others can often observe intermediates of complex networks, why not this one?[21] We postulate that the majority of the intermediates are unstable and transient within the water environment of the reaction, water (which is at its maximum chemical activity as both the solvent and reactant) driving the breaking of any imine formation back to starting materials (in terms of equilibrium chemistry, the water concentration (activity) is unbalancing the equilibrium away from product formation). If there was a template/autocatalytic process occurring with an intermediate and the final product (which clearly has the capacity to cause autoinduction by observation of the seeding process) then we can argue that something observable would be analytically determined so there is a high likelihood of there being no need or possibility for a templated autocatalytic chemical process as part of the imine equilibrium chemistry. Except for the potential of the enol-keto tautomerisation. The chemistry presented in this discussion is well recognised as a non-reversible (normally thermally induced)[21a, 28] reaction that is not the reaction between two or more reactants but simply a conversation between two compounds that normally has a significant kinetic barrier. Enol-keto tautomerisations are normally in equilibrium and for the chemistry presented it has been observed to be different in water than in other chemical environments (not thermally induced and can be made reversible through an acid:base switch in certain circumstances).[21a, 28, 29] Indeed, the chemistry has been used to make materials that are chemically inert to water. For the aniline derivatives we have worked with and published, we do not observe the enol form. Therefore the presence of water and salts (H^+, OH^- and Na^+) are likely enhancing (catalysing) the enol-keto tautomerisation as has been seen in many sugar chemistries (probably the most important autocatalytic process in regards to origin of life chemistries).

In most sugar chemistries that show autocatalytic behaviour (like the Breslow cycle) the products can be reincorporated into the reaction cycle/network. This is not possible for this chemistry and a template mechanism is the option in terms of generating an autocatalytic cycle. This reaction may be catalysed by the intermediate imine interacting with its product (the enol-keto process) with water and the other constituents of the solution resulting in enhanced enol-keto tautomerisation. If we take our linear reaction steps from **A** and **B** to the two intermediates then to the three reacted enol, we could potentially observe the simplest of template-led autocatalytic chemistry for the tautomerisation. However, the product forms supramolecular polymers, and polymerisation is a known mechanism to poisoning a template autocatalysis. Therefore, we think that we can preclude an in-solution template autocatalytic process. And therefore our focus should be put onto how the self-assembly may induce autocatalysis and the observed autoinduction (2) (Figure 3).

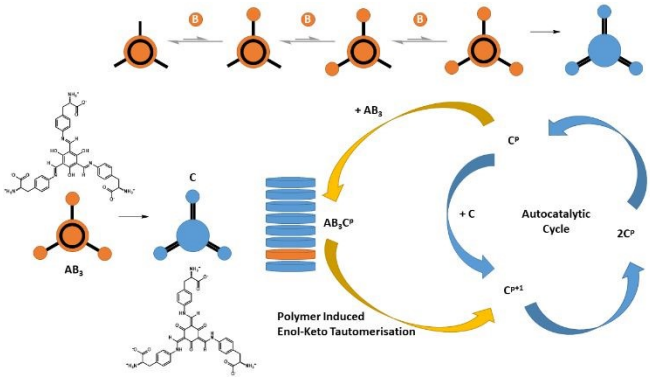

Figure 3. Autoinduction. The product **C** is formed from **AB_3** in a linear reaction pathway of little complexity, with the final reaction of the enol-keto tautomerisation being irreversible. For the kinetics to show sigmoidal rates we suggest that the tautomerisation is coupled to an autocatalytic cycle (autoinduction). The autocatalytic cycle is the supramolecular polymers. We hypothesis that this is coupled to the coassembly of **AB_3** into supramolecular polymers of the final product **C**, thus producing an environment that is favourable to the conversion of **AB_3** to **C** (a polymer induced enol-keto tauomerisation due to the Le Chatelier's principle as the chemical environment would be essentially devoid of water, biasing the equilibrium that is driving **AB_3** back to start materials). It is easy to envision the coassembly of **AB_3** and **C** due to their very similar chemical structures.

Can the self-assembly of supramolecular polymers be autocatalytic in nature? Yes, this is evidenced by over a century of investigations in the nucleation phenomena of crystals and the more recent work on supramolecular polymers like amyloid fibrils.[1, 13] There are two published mechanisms in which the supramolecular polymers in this system could generate autocatalytic kinetics/cycles as part of their self-assembly process. These are: (i) Fragmentation, shearing of individual polymers into one or two "daughters" resulting in an averaged polymer length that is continuously sheared into growing smaller polymers; (ii)

Detachment, secondary nucleation on the surfaces of polymers results in new polymers that are sheared off once a new polymer is of an appropriate size. With these facts in hand, we need now only to couple the self-assembly to the supramolecular polymer to the reaction sequence, thus defining our system as autoinduction autocatalysis (Figure 3).[1] We hypothesis that the simplest way to do this is to couple the irreversible enol-keto tautomerisation to the autocatalytic cycle (conversion of the unstable enol intermediate $\mathbf{AB_3}$ to **C**). The alternative/related mechanism would be the surface functionality of the supramolecular polymer catalysing the formation of **C** via one of the four steps (causing an enhancement of secondary nucleation). This is a possibility but rather complex in nature. A simpler mechanism can be suggested by taking into account that $\mathbf{AB_3}$ and **C** can coassemble into supramolecular polymers as their chemical structures are significantly similar. Once formed, this new chemical environment for $\mathbf{AB_3}$ (out of water) would enhance the probability of it converting to **C** permanently. If we then apply Le Chatelier's principle for the equilibria forming $\mathbf{AB_3}$, we can ascertain that the autocatalytic cycle coupled to the coassembly would dominate the kinetics resulting in our observations, i.e. the rates of inclusion and tautomerisation are greater than the rate of disassembly of the $\mathbf{AB_3C^p}$ polymer. Our final remark is about why **B** is so important to these observations and the reaction network (as similar chemistries we have reported have not been observed to show these autoinducation kinetics). The other similar aniline chemistries we have performed have not had competitive alternative reaction pathways that we see with the alkyl amine and aniline functional groups available in **B**. The other aniline kinetics are far too fast to analytically determine accurately, typically taking mins, but with the reaction competition from the alkyl amine the reaction with **B** is substantially slowed to days and also provides a "convenient" set of products that do not self-assemble, thus slowing nucleation (the induction point). Future work therefore aims to take this competitive chemistry and couple it to other aniline derivatives to determine if this autoinduction can be universally applied to our reactivity of **A**.

Once the reaction had reached completion, the solution was acidified to induce gelation. This resulted in an increase in the concentration of the charge neutral form of $\mathbf{C^{n-}}$ (**C**) which is likely to be part of a more complex supramolecular polymer assembly. This alters the $\mathbf{C^{n-}}$: **C** dynamic ratio with the hydrophobic, zwitterionic species **C** proving to be an effective low molecular weight gelator (LMWG).[31,32] **C** has a critical gel concentration (CGC) of 7.2 mM (0.5 wt%). Acidification of the solution was performed utilising glucono-delta-lactone (GdL) to ensure a homogenous pH change throughout the solution.[33–35] With the isoelectric point (p*I*) of phenylalanine (5.91) being comparable to the apparent p*I* of **C**, it is possible to understand how the addition of GdL induces gelation. At the initial pH of 9, a point which lies above the p*I*, $\mathbf{C^{n-}}$ is the dominant compound (equilibrium) and is the more soluble form. Upon addition of GdL, the pH is slowly lowered below the p*I* of **C**, changing the dynamic ratio towards the more insoluble zwitterion. This insolubility gives rise to the formation of the fibrous network. The discotic nature of **C** lends itself to the one-dimensional assembly of fibres. It is a fibrous morphology that is observed by scanning electron microscopy (SEM) (Fig. S8). It also means there is a reasonable agreement with the cellular solid model for describing the structure of supramolecular gels.[36–38] The cellular solid model predicts that $G' \propto [\text{gelator}]^n$, where G′ is the elastic modulus of the gel and *n* can lie between 1 and 2. By preforming a concentration study and examining the gels responses to an applied oscillatory stress across a range of frequencies, the relationship $G' = 866[\mathbf{C}]^{1.9}$ was obtained (see ESI, section 5). This is in good agreement with the cellular solid model. This relationship allows kinetic data on the chemical reaction that produces $\mathbf{C^{n-}}$ to be extracted from the mechanical properties of the produced gels. Because the reaction is performed in situ, a gel can be set before the final maximum concentration of **C** (or $\mathbf{C^{n-}}$) is reached, provided that [**C**] ≥ CGC. This will result in a gel with a G′ value that corresponds to the specific concentration of **C** achieved at the time of setting. This trapping out of **C** by a mass transfer limitation mechanism allows the mechanical strength of the gel in terms of G′ to be predictably controlled. By changing the time at which the gel is set, under specific conditions of the reaction, we have produced gels from a single stock solution with G′ values ranging from 730 – 2400 Pa. This results in a temporal dependence of the gels mechanical strength with respect to the reaction rate (the rate being autocatalytic in mathematical terms). The rheological measurements can also be utilised to calculate the concentration of **C** using the cellular solid model. Doing so results in a close match in the kinetics determined through rheology to that determined by NMR and HPLC (Figure 1).

Conclusions

In conclusion, we have developed an autoinduction reaction network that can be used predictably to produce a LMWG. This is a highly efficient template based kinetically autocatalytic, self-replicating system by utilizing dynamic imine chemistry coupled to self-assembly. The dynamic nature of the imine bond that allows "error checking" correction gives rise to one reaction product. This product represents a thermodynamic minimum within the systems energy landscape. The self-assembly in solution of the replicator results in the formation of supramolecular polymers which would normally markedly reduce the catalytic efficiency of the system. This inhibiting effect can be overcome by two methods, mechanical agitation of the reaction solution and seeding the solution with a previously isolated sample of replicator. By overcoming the limiting effects of the self-assembly process it was possible to demonstrate exponential growth in replicator concentration. Thus highlighting a connection between the nucleation based self-assembly autocatalytic cycle and the chemical reactions (imine equilibria and enol-keto tautomerisation). Not only does this study provide a basis with which to explore aspects of biochemistry and gelation connected to reaction networks but also the potential of self-replicators playing a crucial role in the origin of life being connected to their supramolecular polymer self-assembly (nucleation based autocatalytic cycles). By controlling the kinetics of the autocatalytic chemical reaction at one end of the hierarchical assembly process we can influence the physical properties of the supramolecular gel at the other. This may have wide ranging applications with in situ formed LMWGs where a specific mechanical strength/formulation is desired.

Author contributions

JSF and GOL provided formal analysis, data curation, investigation, methodology, project administration, validation, visualisation, and writing for this work. In addition, GOL provided supervision,

resources, project administration, funding acquisition, and conceptualisation for this discussion paper.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Heriot-Watt University (JSF PhD funding and fellowship funds for GOL) and the Royal Society of Edinburgh/Scottish Government Fellowship scheme (GOL) are thanked for funding. The University of Lincoln is also thanked for their continued financial and infrastructural support of research. We also thank the many scientists and examiners that we have discussed these observations with, our understanding has been significantly enhanced by these discussions and we hope that has been captured in this discussion paper.

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The data supporting this article have been included as part of the Supplementary Information.

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