nature portfolio

# Peer Review File

# Supramolecular Morphological Transition Driven by Nonequilibrium Break-and-Build Self-Organisation

Corresponding Author: Professor Itaru Hamachi

**This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.**

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Comments to the author
In this communication, R. Kubota et al. report a supramolecular process that creates hierarchical structures across multiple length scales, from micrometers to millimeters. This process occurs under nonequilibrium conditions, in which breaking and building the supramolecular network occur concomitantly, leading to the formation of multiple supramolecular morphologies. Specifically, the system consists of a low-molecular-weight gelator bearing a negative charge and a cationic surfactant. In the absence of the surfactant, the gel consists of ordered fibers. When the surfactant is added, it interacts with the fibrous building blocks, resulting in a nonequilibrium cycle of network breaking and rebuilding. The surfactant concentration controls the final morphology at equilibrium.
The authors elegantly demonstrated the formation of two-dimensional patterns by embedding the fibers in an agarose gel and adding the surfactant in the middle of the gel. This setup results in a propagating wave in which surfactant concentration varies, and several morphologies are observed at different distances from the point of surfactant addition. Finally, the authors demonstrated that complex pattern formation by collision of propagating waves induced by distinct surfactants occurs when they are added at different points of the gel.
The article is methodologically well done with a high degree of detail in chemical characterization, which could be challenging with this kind of system. Furthermore, such supramolecular control can be achieved using relatively simple molecules, such as dipeptides and surfactants, which are likely to be widely available in the scientific community. In view of the experimental results reported here, I encourage this publication to be accepted after addressing some questions.
Related to the experiments.
Q1: In line 142, the authors mention that “This behaviour markedly differs from the dynamic instability induced by anionic SDS micelles.” Is this conclusion related to a previous reference or a control experiment similar to the one described in supplementary Figure 10.
Q2: Section with transient low-temperature pulses. Could the authors comment on the selection of 4 ºC for the pulses, and the frequency of such pulses? Also in this section, could the authors add to the profile analysis in panel D the one in Fig 3d. It will help the reader compare the effect of the temperature.
Q3: Section with two surfactants at the same time. How important is the distance between the point at which surfactants are added? How different are the propagation rates of each surfactant, and how does it affect the collision?
Related to the text
The manuscript is overall very detailed and visual, which helps get the main message at first glance. However, the written part is hard to follow, especially in the section on figures 2 and 3. For example, in lines 177-179, the second layer has inner and outer samples for concentration measurements, which is not obvious from the text. Also, it lacks a micrograph to help the reader follow the text. In Figure 3l, the shaded phase diagram is confusing to read. I find figures 4b-d difficult to understand due to the many overlapping curves and symbols
I miss some important references on nonequilibrium self-assembly applied to materials at these large scales.
https://pubs.acs.org/doi/full/10.1021/jacs.1c13504
https://www.nature.com/articles/ncomms15895
https://onlinelibrary.wiley.com/doi/full/10.1002/anie.202404583
https://www.nature.com/articles/s41586-024-08288-x

Reviewer #2

(Remarks to the Author)

In this manuscript, the authors report a supramolecular system in which peptide-based nanofibers undergo growth and shrinkage upon interaction with surfactant micelles. Building on their previous work (JACS 2024), the authors investigate how interactions between Ox peptide nanofibers and SDS micelles lead to long-lived autonomous dynamics, including growth, shrinkage, and treadmilling-like behavior of fibers observed by confocal microscopy. The authors interpret this as a type of "synthetic dynamic instability," analogous to biological filament systems such as microtubules.
The phenomenon itself is interesting and potentially significant, especially from the perspective of nonequilibrium supramolecular systems and reaction–diffusion phenomena. The experimental observations, and in particular the long-lived growth/shrinkage dynamics observed via time-lapse confocal microscopy, are compelling and appear internally consistent with the authors' proposed mechanism involving micelle-mediated fiber disassembly and re-nucleation.
However, in its current form the manuscript tends to oversell the interpretation of the phenomenon as morphogenesis, and the mechanistic interpretation remains largely plausible rather than experimentally demonstrated. In my opinion, the work needs both conceptual clarification and additional experimental support before publication.

Major Comments to be addressed:

A) The manuscript repeatedly frames the phenomenon as pattern formation or morphogenesis, which I find misleading. In classical nonlinear systems, pattern formation refers to the emergence of spatially periodic structures, typically arising from instabilities such as Liesegang patterns, Turing patterns, Belousov–Zhabotinsky waves, or viscous fingering. These systems produce repeating domains or periodic spatial structures. By contrast, the system presented here does not appear to produce spatially periodic patterns. Instead, it shows localized growth and shrinkage of supramolecular fibers driven by micelle–fiber interactions, resulting in dynamic restructuring of the fiber network.
I therefore strongly suggest that the authors remove or substantially tone down the claims of pattern formation or morphogenesis and instead describe the system more accurately as a reaction–diffusion process coupled to supramolecular self-assembly that leads to dynamic restructuring of nanofiber networks.

B) The authors propose a mechanism in which:
1) SDS micelles interact with nanofiber tips.
2) The hydrophobic micelle core promotes fiber disassembly.
3) Short peptide aggregates are released from micelles.
4) These aggregates act as seeds for regrowth.
5) Repeated cycles of micelle interaction produce growth/shrinkage dynamics.
This mechanism seems reasonable, but at present it appears largely inferred rather than experimentally demonstrated.
For example, the key mechanistic steps as listed above are not directly probed.

Additional experiments that would help in this regard:

B1) Mixing experiments using different SDS concentrations with preformed Ox fibers to test whether coexisting morphologies appear in equilibrium.

B2) Experiments examining micelle–fiber interactions more directly (e.g., fluorescence labeling or scattering techniques).

B3) Measurements to determine whether peptide material becomes temporarily sequestered within micelles.

In its current form, the rational remains too much in the form of story-telling in my opinion.

C) Others in the field have already tried to frame artificial processes to morphogenesis. See for example the book chapter by Estevez-Torres and co-workers (DOI: 10.1002/9783527821990.ch2) which frames morphogenesis and pattern formation using concepts from embryo development, where pattern formation refers to the emergence of spatial concentration patterns generated by reaction–diffusion dynamics, while morphogenesis specifically denotes shape changes driven by chemomechanical coupling and force generation in materials.

What the authors observe—i.e., localized growth and shrinkage of supramolecular fibers mediated by micelle–fiber interactions—does not correspond to classical reaction–diffusion pattern formation (which produces periodic or spatially organized concentration structures) nor to morphogenesis (which involves mechanically driven shape changes of a material). Instead, the results are better interpreted as a reaction–diffusion–coupled supramolecular assembly process that generates dynamic restructuring of a fiber network without forming a periodic chemical pattern or inducing macroscopic morphogenetic deformation. The authors could adopt the terminology used in the chapter I mentioned above, by clearly distinguishing between (i) reaction–diffusion processes that produce spatial chemical patterns, (ii) supramolecular self-assembly dynamics that restructure material organization, and (iii) true morphogenetic processes requiring chemomechanical coupling. By positioning their system in the second category, the authors avoid introducing confusing terminology while still highlighting its relevance as a synthetic nonequilibrium material in which reaction–diffusion chemistry modulates supramolecular assembly.

Reviewer #3

(Remarks to the Author)
The results described in the paper use an anionic peptide-based small molecule hydrogelator that assembles into a fibrous network. Diffusion of a cationic surfactant into this network leads to dissolutions and subsequent re-assembly of different fiber structures depending on surfactant structure, concentration and time. This concept is then used to create hydrogels with spatially different patterns. This concept is new and goes beyond the state of the art, in particular in providing access to multiple different structures from a single mixture of components. In this sense, the analogy to cellular morphogenesis holds. I do wonder about the use of “nonequilibrium” in this case, in what way is it nonequilibrium? Are the observed structures in fact at equilibrium under the conditions where they appear (i.e. at the surfactant concentration at that location and moment) or are these transient structures and will the change into something else over time spontaneously? The phase diagram in figure 2e suggests that these are end points under the conditions and that it is the changing gradient of the surfactant that is the non-equilibrium part here. Anyway, besides some minor points listed below and some unclarity about terminology, this is a high quality and novel piece of work that deserves to be published.

Other points:
“Considering these experimental results, we propose a plausible mechanism for the…” and “Using the spatiotemporal imaging and structural analysis described above, we propose a plausible mechanism for the…” I find the use of “plausible” somewhat problematic, and I think it could simply be removed. Plausible is an assessment, and that should be up to the reader. Also, would one ever suggest a non-plausible (in one’s own assessment) mechanism?

“we next sought to apply the break-and-build dynamics to autonomous pattern formation in two dimensions.” What does “autonomous” mean in this context? That it is not determined by an external field or pre-imposed pattern? Because the location, shape and diameters of the initial injection of reagents do influence this quite a bit, so I’m not sure this is really autonomous. Unless the term is defined more sharply.

A Liesegang pattern (much simpler than in the current work) was also observed in the paper on reaction-diffusion by Eelkema and van Esch (Nat. Commun. 2017, 8, 15317).

“Overall, brief low-temperature pulses can preferentially modulate the spatial patterning of the thick coassembled fibres during the self-organising process.” Very interesting, any idea what the underlying mechanism is? Is it a change in CMC, or in the interaction between the surfactant and gelator, or something else?

“Despite recent advances, multicomponent systems rarely extend beyond tens of micrometres, and few studies have achieved the spatial organisation of such supramolecules at the macroscale, as demonstrated here (Supplementary Fig. 15).” The value of this statement very much depends on a number of definitions or boundaries. In the reaction-diffusion field (covered by David Smith, ref. 19) macroscopic (mm-cm) structures are regularly made. Similarly when using light and light-switchable gelators or for instance photoacids (see e.g. Adams, Eelkema) or patterned surfaces.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
As my comments have been mostly ignored or misinterpreted, I maintain my stance that the work is not suitable for publication. There is no evidence of biological morphogenesis, and it is therefore an unsuitable analogy and invokes excitement, but falls flat beyond the title.

"We thus refer to this process as supramolecular morphogenesis."

This is exactly what I meant by an overstatement. You are introducing a new word that sounds fancy to describe a process that already has a word: a morphological transition.
The morphogen is simply a chemical trigger for the morphological transitions.

I leave it up to the editors to decide what to do with that, and do not need to see another response (given that this is going to end up in a sementaical back and forth and the authors showed no interest in addresing my previous comments)

Reviewer #2

(Remarks to the Author)
All my concerns have been reasonably addressed.

Reviewer #3

(Remarks to the Author)
the authors have thoroughly addressed the points raised by the referees, and I'm now happy to recommend the manuscript for publication. I'd like to congratulate the authors on these exciting results.

**Modification in the main text:**

Page 1, line 4:

Ryou Kubota[1,2,*], Yuriki Ikuta[1], Shogo Torigoe[1], Naoki, Kanazawa[2], Itaru Hamachi[1,3,4,*]

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[2]Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 744 Moto-oka, Nishi-ku, Fukuoka 819-0395, Japan.
[3]Institute of Advanced Energy, Kyoto University, Uji, 611-0011, Japan.
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**Request 1:**
Your work characterises chemical or biomolecular materials. Please see the link below for reporting requirements. There is no form to upload but you may need to revise your manuscript to comply with this policy.
https://www.nature.com/ncomms/submit/chemical-characterisation

**Reply:**
A $^{1}H$ NMR spectrum of NBD-TA was newly added in the Supplementary Information (supplementary Fig. 21). We have tried to measure its $^{13}C$ NMR, but the signal intensity is too weak to identify all the signals assigned to NBD-TA.

**Request 2:**
Please consider the needs of colourblind readers (a substantial minority of the male population) when choosing colours for figures. Many colourblind readers cannot interpret visuals that rely on discrimination of green and red, for example. Thus, we ask you to recolour green-and-red heatmaps, graphs and schematics for which colours are chosen arbitrarily. Recolouring primary data, such as fluorescence or rainbow pseudo-coloured images, to colour-safe combinations such as green and magenta, turquoise and red, yellow and blue or other accessible colour palettes is strongly encouraged.

**Reply:**
We recoloured all of the CLSM images to a combination of green and magenta.

**Request 3:**
All Nature Communications manuscripts must include a “Data Availability” section after the Methods section but before the References. If any of the data can only be shared on request or are subject to restrictions, please specify the reasons and explain how, when, and by whom the data can be accessed. For more information on this policy and a list of examples, see:
https://www.nature.com/documents/nr-data-availability-statements-data-citations.pdf

As Nature Portfolio policies strongly encourage you to share your research data in a public repository (e.g. spreadsheets, text, images), we are partnering with the figshare

repository so that you can use the figshare integration via the 'Research Data Deposition' tab when submitting your revised manuscript.

Data are stored privately until a manuscript decision is reached and you can edit/withdraw them up to this point: you retain rights and control over your data. The data will be published at the same time as your article; you will receive a data DOI, with guidance on linking the data and manuscript. In the event your manuscript is not accepted, you can keep or remove your data in figshare.

We recommend the use of discipline-specific repositories where available and for a number of data typesthis is mandatory. Ensure you do not submit these data types or any sensitive data to figshare.

We strongly encourage you to deposit all new data associated with the paper in a persistent repository where they can be freely and enduringly accessed. We recommend submitting the data to discipline-specific and community-recognised repositories; a list of repositories is provided here: http://www.nature.com/sdata/policies/repositories
Refer to our data policies here: https://www.nature.com/nature-portfolio/editorial-policies/reporting-standards#availability-of-data

To maximise the reproducibility of research data, we ask that you provide a Source Data file containing the raw data underlying the following types of display items:
- Any reported means/averages in box plots, bar charts, and tables
- Dot plots/scatter plots, especially when there are overlapping points
- Line graphs
- Uncropped and unprocessed scans of all blots and gels including all quantified replicates. The edge of membranes, molecular weight ladders and loading controls should be presented on all blots. Where membranes have been cut, please ensure that at least one marker above and below is present. For an example of presentation of full scan blots, see the Source Data file of https://www.nature.com/articles/s41467-020-16984-1#Sec35 and for more information, please refer to https://www.nature.com/nature-research/editorial-policies/image-integrity. The data should be provided in a single Excel file with data for each figure/table in a separate sheet, or in multiple labelled files within a zipped folder. Name this file or folder 'Source Data', and include a brief description in your cover letter. The "Data Availability" section should also include the statement "Source Data are provided with this paper." To learn more about our motivation behind this policy, please see: https://www.nature.com/articles/s41467-018-06012-8. A Source Data file is not necessary if all display items presented in the main manuscript and supplementary information can be reproduced from raw data and code that have already been shared in a public repository.

**Reply:**
We added the Data Availability Section in the main text and provided Source Data file as an excel format.

**Modification in the main text:**
Page 23, line 480
**Data Availability**
Additional data to support the findings of this study are provided in the Supplementary Information. Source data are available with this paper.

**Request 4:**
Please replace your bar graphs with plots that feature information about the distribution of the underlying data. All data points should be shown for plots with a sample size less than 10. For larger sample sizes, please consider box-and-whisker or violin plots as alternatives. Measures of centrality, dispersion and/or error bars should be plotted and described in the figure legend.

**Reply:**
We modified Figure 3i from violin and dot plot to violin and box plot.

**Modification in the main text:**
Page 15, line 351:
i. Violin and box plots of fibre widths. The white dots represent the median, and the boxes indicate the interquartile range.

## *Response to Reviewer 1*

**Reviewer 1's comments:**

In this communication, R. Kubota et al. report a supramolecular process that creates hierarchical structures across multiple length scales, from micrometers to millimeters. This process occurs under nonequilibrium conditions, in which breaking and building the supramolecular network occur concomitantly, leading to the formation of multiple supramolecular morphologies. Specifically, the system consists of a low-molecular-weight gelator bearing a negative charge and a cationic surfactant. In the absence of the surfactant, the gel consists of ordered fibers. When the surfactant is added, it interacts with the fibrous building blocks, resulting in a nonequilibrium cycle of network breaking and rebuilding. The surfactant concentration controls the final morphology at equilibrium.

The authors elegantly demonstrated the formation of two-dimensional patterns by embedding the fibers in an agarose gel and adding the surfactant in the middle of the gel. This setup results in a propagating wave in which surfactant concentration varies, and several morphologies are observed at different distances from the point of surfactant addition. Finally, the authors demonstrated that complex pattern formation by collision of propagating waves induced by distinct surfactants occurs when they are added at different points of the gel.

The article is methodologically well done with a high degree of detail in chemical characterization, which could be challenging with this kind of system. Furthermore, such supramolecular control can be achieved using relatively simple molecules, such as dipeptides and surfactants, which are likely to be widely available in the scientific community. In view of the experimental results reported here, I encourage this publication to be accepted after addressing some questions.

**Reply:**

We deeply appreciated your positive comments. According to your comments, we amended our manuscript as shown below. All modifications are highlighted in red letters. We believe that the quality of modified manuscript is suitable for publication in *Nature Communications*.

**Comment 1**: In line 142, the authors mention that "This behaviour markedly differs from the dynamic instability induced by anionic SDS micelles." Is this conclusion

related to a previous reference or a control experiment similar to the one described in supplementary Figure 10.

**Reply:**
Thank you very much for your comments. This conclusion is related to our previous paper. To prevent any misunderstanding by readers, we clarified this sentence and added a reference.

**Modification in the main text:**
Page 5, line 146:
This behaviour differs from the dynamic instability induced by anionic SDS micelles, which is characterised by stochastic fluctuations in fibre growth and shrinkage[35]. The dynamics observed here fundamentally manifests as a multistep structural transition, albeit with the potential for transient, localized dynamic instability-like behaviours.

**Comment 2**: Section with transient low-temperature pulses. Could the authors comment on the selection of 4 ºC for the pulses, and the frequency of such pulses? Also in this section, could the authors add to the profile analysis in panel D the one in Fig 3d. It will help the reader compare the effect of the temperature.

**Reply:**
We appreciate your comments on the low temperature pulse experiments. The reason we select 4 ºC was to use a fridge for temperature control. Regarding the frequency of the pulse, we chose the pulse times, corresponding to when the leading edge (the dark region) of propagating waves reached the positions of the 2nd, 3rd, and 4th rings at 18 h.

According to your comment, we newly add the overlay profile analysis of the bright field images with/without low temperature pulses shown in supplementary Fig. 12a.

**Modification in the main text:**
Page 8, line 253:
Brief low-temperature pulses (4°C for 1 min) were applied 30 min, 1 h, and 2 h after the addition of DTAC, corresponding to when the leading edge (the dark region) of the propagating wave reached the positions of the 2nd, 3rd and 4th rings at 18 h,

respectively (Fig. 5a). The macroscopic and BF images revealed the emergence of new dark rings located near the centres of the 2nd and 3rd rings (Fig. 5b–d, Supplementary Fig. 12).

**Comment 3:** Section with two surfactants at the same time. How important is the distance between the point at which surfactants are added? How different are the propagation rates of each surfactant, and how does it affect the collision?

**Reply:**

We deeply appreciate your constructive suggestions. We have performed additional collision experiments at different hole distances. As a result, the collision patterns were found to strongly depend on the hole distance. When the holes were spaced 2.6 mm apart, the mixed opaque regions formed at both the top and bottom of the region between the holes (supplementary Fig. 17). In contrast, no collision was observed at a distance of 8.2 mm (supplementary Fig. 18).

Regarding the propagation rates of the surfactants, we analyzed their velocities based on the dynamics of the dark region in Alexa647 channel. The initial propagation velocities were 110 μm/min for DTAC and 71 μm/min for TTAC, demonstrating that DTAC exhibited a higher propagation velocity than TTAC (supplementary Fig. 16). Furthermore, the distances from the injection points to the collision point was measured at 4611 μm for DTAC and 3621 μm for TTAC. These quantitative results confirm that the collision occurred closer to the TTAC injection point, reflecting the difference in their propagation rates. To explain these results, we modified the main text and supplementary information as follows.

**Modification in the main text:**

Page 9, line 284:

Quantitative analysis indicated that the initial propagation velocities were 110 μm/min for DTAC and 71 μm/min for TTAC, demonstrating that DTAC exhibited a higher propagation velocity than TTAC (Supplementary Fig. 16). This difference caused the collision to occur closer to the TTAC injection point (with distances of 4611 and 3621 μm from the respective injection points). Furthermore, we observed that the collision patterns strongly depended on the distance between the two holes. When the holes were spaced 2.6 mm apart, the mixed opaque regions were produced at both the top and bottom of the region between the holes (Supplementary Fig. 17). In contrast, no

collision was observed at a distance of 8.2 mm (Supplementary Fig. 18).

**Modification in the supplementary information:**
Page 23–26: We newly added supplementary Fig. 16–18.

**Comment 4**: The manuscript is overall very detailed and visual, which helps get the main message at first glance. However, the written part is hard to follow, especially in the section on figures 2 and 3. For example, in lines 177-179, the second layer has inner and outer samples for concentration measurements, which is not obvious from the text. Also, it lacks a micrograph to help the reader follow the text. In Figure 3I, the shaded phase diagram is confusing to read. I find figures 4b-d difficult to understand due to the many overlapping curves and symbols.

**Reply:**
Thank you very much for your suggestion. According to your comment, we revised the main text and figures as follows:

1. We added an explanation in the main text and provided an additional image in the supplementary information (supplementary Fig. 10) to clarify the inner and outer sampling position for the concentration analysis.
2. For Figure 3l, we increased the transparency of the background phase diagram to enhance its clarity.
3. Regarding Figure 4b-d, we reduced the numbers of overlapping curves.

**Modifications in the main text:**
Page 6, line 182:
The patterned hydrogel was cut into individual rings, with the 2nd ring further divided into the inner and outer regions, and each gel piece was dissolved in dimethyl sulfoxide-$d_6$ for analysis (Supplementary Fig. 9, 10).

**Modification in the supplementary information:**
Page 17: we added supplementary Fig. 10.

**Comment 5:** I miss some important references on nonequilibrium self-assembly applied to materials at these large scales.

https://pubs.acs.org/doi/full/10.1021/jacs.1c13504
https://www.nature.com/articles/ncomms15895
https://onlinelibrary.wiley.com/doi/full/10.1002/anie.202404583
https://www.nature.com/articles/s41586-024-08288-x

**Reply:**
According to your suggestion, we added the suggested references.

## *Response to Reviewer 2*

**Reviewer 2's comments**

In this manuscript, the authors report a supramolecular system in which peptide-based nanofibers undergo growth and shrinkage upon interaction with surfactant micelles. Building on their previous work (JACS 2024), the authors investigate how interactions between Ox peptide nanofibers and SDS micelles lead to long-lived autonomous dynamics, including growth, shrinkage, and treadmilling-like behavior of fibers observed by confocal microscopy. The authors interpret this as a type of "synthetic dynamic instability," analogous to biological filament systems such as microtubules.

The phenomenon itself is interesting and potentially significant, especially from the perspective of nonequilibrium supramolecular systems and reaction–diffusion phenomena. The experimental observations, and in particular the long-lived growth/shrinkage dynamics observed via time-lapse confocal microscopy, are compelling and appear internally consistent with the authors' proposed mechanism involving micelle-mediated fiber disassembly and re-nucleation.

However, in its current form the manuscript tends to oversell the interpretation of the phenomenon as morphogenesis, and the mechanistic interpretation remains largely plausible rather than experimentally demonstrated. In my opinion, the work needs both conceptual clarification and additional experimental support before publication.

**Reply:**

We appreciate your comments on our manuscript. We would like to emphasize our claim and scope of this manuscript, as there appear to be a slight misunderstanding regarding the difference between our current study and our previous work. In our previous *JACS* paper, we reported autonomous growth and shrinkage of peptide fibers induced by addition of SDS micelles, as you mentioned. In sharp contrast, the current manuscript reports a distinct system using cationic surfactants to achieve ***macroscopic, reaction-diffusion-driven spatial patterning***, as demonstrated in Figure 3 and 4, which was not explored in the previous paper.

The main objective of this study is to demonstrate that the interaction between the peptide fibers and *cationic* surfactants drives a break-and-build dynamics, leading to the formation of new coassembled fibers. ***By coupling this unique fiber dynamics with a time-dependent surfactant concentration gradient, we successfully achieved reaction-diffusion-dependent concentric spatial pattern formation***, which is the central discovery of this work.

Regarding your concern about overselling the interpretation of morphogenesis, we used this term because we found an interesting analogy to biological tissue formation. In biological systems, morphogenesis is dynamically driven by the orchestration of the sequential differentiation of stem/progenitor cells (construction) and localized apoptosis (deconstruction), which together shape complex tissue architectures. As described in the introduction, our synthetic system operates on the similar concept. The spatiotemporal gradient driven by morphogen (surfactant) diffusion governs the decomposition of progenitor fibers (analogous to localized apoptosis) and the subsequent positional information of differentiated coassembled fibers (analogous to cell differentiation) through the break-and-build dynamics. This correspondence proves that our reaction-diffusion-driven patterning is a synthetic analogue to biological tissue development. Therefore, we believe that the term "supramolecular morphogenesis" is not an overstatement. To emphasize the conceptual similarity, we revised the main text as shown below.

**Modification in the main text:**

Page 3, line 74:

These synthetic break-and-build events correspond to localized apoptosis and differentiation behaviors, respectively. We thus refer to this process as supramolecular morphogenesis.

Page 5, line 146:

This behaviour differs from the dynamic instability induced by anionic SDS micelles, which is characterised by stochastic fluctuations in fibre growth and shrinkage[35]. The dynamics observed here fundamentally manifests as a multistep structural transition, albeit with the potential for transient, localized dynamic instability-like behaviours.

**Major Comments to be addressed:**

**Comment A**

The manuscript repeatedly frames the phenomenon as pattern formation or morphogenesis, which I find misleading. In classical nonlinear systems, pattern formation refers to the emergence of spatially periodic structures, typically arising from instabilities such as Liesegang patterns, Turing patterns, Belousov–Zhabotinsky waves, or viscous fingering. These systems produce repeating domains or periodic spatial structures. By contrast, the system presented here does not appear to produce spatially

periodic patterns. Instead, it shows localized growth and shrinkage of supramolecular fibers driven by micelle–fiber interactions, resulting in dynamic restructuring of the fiber network.

I therefore strongly suggest that the authors remove or substantially tone down the claims of pattern formation or morphogenesis and instead describe the system more accurately as a reaction–diffusion process coupled to supramolecular self-assembly that leads to dynamic restructuring of nanofiber networks.

**Reply:**
As replied to your general comment, the break-and-build dynamics coupled with morphogen diffusion successfully generates distinct, macroscopic concentric ring patterns at the millimetre scale. These ring patterns exhibit discontinuous boundaries and well-defined, spatially segregated periodic domains of different fibre types and densities. The real-time observation of multiple propagating waves further demonstrates that this is a reaction-diffusion process. Therefore, this phenomenon goes far beyond localized network restructuring and clearly represents a macroscopically-organized spatial pattern analogous to periodic precipitation.

**Comment B**
The authors propose a mechanism in which:
1) SDS micelles interact with nanofiber tips.
2) The hydrophobic micelle core promotes fiber disassembly.
3) Short peptide aggregates are released from micelles.
4) These aggregates act as seeds for regrowth.
5) Repeated cycles of micelle interaction produce growth/shrinkage dynamics.
This mechanism seems reasonable, but at present it appears largely inferred rather than experimentally demonstrated.
For example, the key mechanistic steps as listed above are not directly probed.

**Comment B1**: Mixing experiments using different SDS concentrations with preformed Ox fibers to test whether coexisting morphologies appear in equilibrium.

**Reply:**
According to the reviewer's comment, we newly conducted experiments using different SDS concentrations. As shown in supplementary Fig. 11, no significant spatial patterns

emerge at any SDS concentrations we tested.

**Modification in the supplementary information:**
Page 18: we added new data in supplementary Fig. 11.

**Comment B2**: Experiments examining micelle–fiber interactions more directly (e.g., fluorescence labeling or scattering techniques).
**Comment B3**: Measurements to determine whether peptide material becomes temporarily sequestered within micelles.

**Reply:**
We deeply appreciate your insightful suggestion. Regarding the synthetic dynamic instability (related to our previous *JACS* paper), we have attempted to investigate the detailed mechanisms by using fluorescence labeling and electron microscopic techniques, as you mentioned. Since the mechanism of dynamic instability is out-of-scope of this paper, we will report the mechanism in the near future.

In its current form, the rational remains too much in the form of story-telling in my opinion.
**Comment C**
Others in the field have already tried to frame artificial processes to morphogenesis. See for example the book chapter by Estevez-Torres and co-workers (DOI: 10.1002/9783527821990.ch2) which frames morphogenesis and pattern formation using concepts from embryo development, where pattern formation refers to the emergence of spatial concentration patterns generated by reaction–diffusion dynamics, while morphogenesis specifically denotes shape changes driven by chemomechanical coupling and force generation in materials.

What the authors observe—i.e., localized growth and shrinkage of supramolecular fibers mediated by micelle–fiber interactions—does not correspond to classical reaction–diffusion pattern formation (which produces periodic or spatially organized concentration structures) nor to morphogenesis (which involves mechanically driven shape changes of a material). Instead, the results are better interpreted as a reaction–diffusion–coupled supramolecular assembly process that generates dynamic restructuring of a fiber network without forming a periodic chemical pattern or inducing

macroscopic morphogenetic deformation. The authors could adopt the terminology used in the chapter I mentioned above, by clearly distinguishing between (i) reaction–diffusion processes that produce spatial chemical patterns, (ii) supramolecular self-assembly dynamics that restructure material organization, and (iii) true morphogenetic processes requiring chemomechanical coupling. By positioning their system in the second category, the authors avoid introducing confusing terminology while still highlighting its relevance as a synthetic nonequilibrium material in which reaction–diffusion chemistry modulates supramolecular assembly.

**Reply:**

As noted in our response to your general comment, we successfully achieved reaction-diffusion-dependent concentric spatial pattern formation driven by coupling the unique fibre dynamics with a surfactant concentration gradient. We would like to claim that our system satisfies Estevez-Torres's definition of pattern formation (the emergence of spatial concentration patterns generated by reaction–diffusion dynamics).

Furthermore, we again emphasize that the term "morphogenesis" is appropriate. We use it to imply the synthetic analogue to the core biological essence of morphogenesis: the process of organization through cell death and differentiation in accordance with morphogen concentration gradients. To avoid ambiguity for the readers, we have incorporated this definition into the introduction of the revised manuscript.

**Modification in the main text:**

Page 3, line 74:

These synthetic break-and-build events correspond to localized apoptosis and differentiation behaviors, respectively. We thus refer to this process as supramolecular morphogenesis.

## *Response to Reviewer 3*

**Reviewer 3's comments:**

The results described in the paper use an anionic peptide-based small molecule hydrogelator that assembles into a fibrous network. Diffusion of a cationic surfactant into this network leads to dissolutions and subsequent re-assembly of different fiber structures depending on surfactant structure, concentration and time. This concept is then used to create hydrogels with spatially different patterns. This concept is new and goes beyond the state of the art, in particular in providing access to multiple different structures from a single mixture of components. In this sense, the analogy to cellular morphogenesis holds. I do wonder about the use of "nonequilibrium" in this case, in what way is it nonequilibrium? Are the observed structures in fact at equilibrium under the conditions where they appear (i.e. at the surfactant concentration at that location and moment) or are these transient structures and will the change into something else over time spontaneously? The phase diagram in figure 2e suggests that these are end points under the conditions and that it is the changing gradient of the surfactant that is the non-equilibrium part here. Anyway, besides some minor points listed below and some unclarity about terminology, this is a high quality and novel piece of work that deserves to be published.

**Reply:**

We deeply appreciate your positive comments. As you pointed out, the term "nonequilibrium" is used to describe the transient break-and-build fiber dynamics (including the propagating waves) that occur prior to reaching a steady state. These dynamics are driven by the time-dependent changes in the local concentration of the surfactant. In contrast, the resultant concentric patterns are formed by 18 h and show negligible changes thereafter, implying that they are in or near thermodynamic equilibrium (or a metastable state). Therefore, we do not describe these concentric patterns as "nonequilibrium."

According to your other comments, we amended our manuscript as shown below. We believe that the quality of modified manuscript is suitable for publication in *Nature Communications*.

**Modifications in the main text:**

Page 5, line 163:

The resultant concentric patterns showed negligible changes thereafter, implying that

they can be regarded as thermodynamically stable or metastable states.

**Comment 1:**
"Considering these experimental results, we propose a plausible mechanism for the…" and "Using the spatiotemporal imaging and structural analysis described above, we propose a plausible mechanism for the…" I find the use of "plausible" somewhat problematic, and I think it could simply be removed. Plausible is an assessment, and that should be up to the reader. Also, would one ever suggest a non-plausible (in one's own assessment) mechanism?

**Reply:**
According to your comment, we deleted a word, "plausible", from the main text.

**Modification in the main text:**
Page 5, line 135:
Considering these experimental results, we propose a ~~plausible~~ mechanism for the nonequilibrium break-and-build dynamics in Fig. 2f.

Page 8, line 232:
Using the spatiotemporal imaging and structural analysis described above, we propose a ~~plausible~~ mechanism for the emergence of the two-dimensional concentric patterns in Fig. 4f.

Page 13, line 335:
**f**. A ~~Plausible~~ mechanism of break-and-build cycles from Ox fibres to thin and thick coassembled fibres, followed by fibre decomposition.

Page 16, line 372:
**f**. A ~~Plausible~~ mechanism of the supramolecular morphogenesis

**Comment 2:**
"we next sought to apply the break-and-build dynamics to autonomous pattern formation in two dimensions." What does "autonomous" mean in this context? That it is not determined by an external field or pre-imposed pattern? Because the location, shape

and diameters of the initial injection of reagents do influence this quite a bit, so I'm not sure this is really autonomous. Unless the term is defined more sharply.

**Reply:**
To address your suggestion, we deleted a word "autonomous" from the main text to avoid any ambiguity.

**Modification in the main text:**
Page 5, line 151:
~~**Autonomous**~~ **Spatial pattern formation in two dimensions**
With the unique break-and-build dynamics in hand, we next sought to apply the break-and-build dynamics to ~~autonomous~~ pattern formation in two dimensions.

Page 8, line 243:
Overall, the ~~autonomous~~ spatial patterning observed in this system can be ascribed to the interplay between the DTAC diffusion-driven concentration gradient and the nonequilibrium break-and-build dynamics comprising supramolecular fibre formation and decomposition.

Page 9, line 294:
These results demonstrate that the ~~autonomous~~ pattern formation is strongly affected by the type of cationic surfactant, probably the hydrophobicity and self-assembly properties (*e.g.*, CMC) of the surfactant, and the resulting pattern can be further modulated by the interactions between propagating waves generated by surfactant diffusion.

Page 11, line 320:
**c**. Supramolecular morphogenesis: ~~autonomous~~ formation of a macroscopic concentric structure composed of distinct types of supramolecular fibres, which are generated through nonequilibrium break-and-build dynamics.

Page 22, line 440:
~~**Autonomous**~~ **Concentric patterning in a composite hydrogels.**

Page 22, line 454:
The hydrogel was prepared according to the protocol similar to described in

"~~Autonomous~~ Concentric patterning in a composite hydrogels",

**Comment 3:**

A Liesegang pattern (much simpler than in the current work) was also observed in the paper on reaction-diffusion by Eelkema and van Esch (Nat. Commun. 2017, 8, 15317).

**Reply:**

We appreciate your comment. In response to your suggestion, we added one sentence in the main text.

**Modification in the main text:**

Page 2, line 60:

For example, nonlinear spatial patterns similar to Liesegang ring have been achieved by supramolecular fibre s[21,30]

**Comment 4:**

"Overall, brief low-temperature pulses can preferentially modulate the spatial patterning of the thick coassembled fibres during the self-organising process." Very interesting, any idea what the underlying mechanism is? Is it a change in CMC, or in the interaction between the surfactant and gelator, or something else?

**Reply:**

We appreciate your insightful questions regarding the underlying mechanism of low temperature pulse. As discussed in the main text, these pulses have a significant impact on the formation of thick coassembled fibers, but not on thin coassembled fibers. This finding implies that the low temperature influences the nucleation processes and/or the stability of thick coassembled fibers. Although a comprehensive determination of the exact mechanism (including CMC changes) is currently beyond the scope of this paper, we will plan to investigate these underlying principles in detail in a future study.

**Comment 5:**

"Despite recent advances, multicomponent systems rarely extend beyond tens of micrometres, and few studies have achieved the spatial organisation of such supramolecules at the macroscale, as demonstrated here (Supplementary Fig. 15)." The value of this statement very much depends on a number of definitions or boundaries. In

the reaction-diffusion field (covered by David Smith, ref. 19) macroscopic (mm-cm) structures are regularly made. Similarly when using light and light-switchable gelators or for instance photoacids (see e.g. Adams, Eelkema) or patterned surfaces.

**Reply:**

We agree with the reviewer that macroscopic structures have been successfully achieved in fields such as reaction-diffusion and light-driven systems. However, we would like to emphasize that our study focuses on the simultaneous spatial orchestration of *multiple distinct supramolecular species*, which remains a significant challenge compared to the predominantly single-component or co-assembled patterns reported in the suggested literatures. To address the reviewer's comment, we modified the main text and newly incorporated the suggested references.

**Modification in the main text:**

Page 10, line 308:

While the macroscopic spatial control of a single type of supramolecular assembly has been extensively investigated[20,42,54,55], the orchestrated patterning of multicomponent systems rarely extends beyond tens of micrometres[56]. The spatial organisation of multiple distinct types of supramolecules at the macroscale from the bottom up remains a challenge, as demonstrated here (Supplementary Fig. 20).

## *Response to Reviewer 1*

**Reviewer 1's comments:**

As my comments have been mostly ignored or misinterpreted, I maintain my stance that the work is not suitable for publication. There is no evidence of biological morphogenesis, and it is therefore an unsuitable analogy and invokes excitement, but falls flat beyond the title.

"We thus refer to this process as supramolecular morphogenesis."

This is exactly what I meant by an overstatement. You are introducing a new word that sounds fancy to describe a process that already has a word: a morphological transition. The morphogen is simply a chemical trigger for the morphological transitions.

I leave it up to the editors to decide what to do with that, and do not need to see another response (given that this is going to end up in a sementaical back and forth and the authors showed no interest in addresing my previous comments)

**Reply:**

As suggested by the editor and this reviewer, we have changed the term "supramolecular morphogenesis" to "supramolecular morphological transition." We also removed the sentences that describe our systems an analogy to biological systems. We believe that the quality of modified manuscript is suitable for publication in *Nature Communications*. All modifications are highlighted by red letters.

**Modifications in the main text:**

Page 1, title:

**Supramolecular Morphological Transition Driven by Nonequilibrium Break-and-Build Self-Organisation**

Page 1, line 24:

Here, we present a supramolecular morphological transition that enables spatial control over the differentiation and decomposition of synthetic self-assembled fibres.

Page 2, line 68:

Bottom-up strategies ~~analogous to biological morphogenesis~~ which enable the

positional formation of multiple supramolecular assemblies into macroscopic, spatially segregated architectures, remain largely undeveloped.

Page 2, line 71:
Drawing inspiration from biological morphogenesis, herein, we introduce the concept of supramolecular morphological transition (Fig. 1c).

Page 2, line 83:
~~These synthetic break-and-build events correspond to localized apoptosis and differentiation behaviors, respectively. We thus refer to this process as supramolecular morphogenesis.~~

Page 10, line 320:
This system provides a strategy for spatially patterning artificial soft materials, ~~reminiscent of biological morphogenesis,~~ where progenitor peptide fibres differentiate into coassembled fibres in response to a morphogen gradient, and the resulting patterns can be modulated by using multiple morphogens.

Page 18, line 561:
**Figure 1. Concept of a supramolecular morphological transition.**
**a**,**b**. Schematic illustrations of (**a**) biological morphogenesis and (**b**) the Liesegang phenomenon, synthetic periodic precipitation patterns. **c**. Supramolecular morphological transition:

Page 22, line 590:
**Figure 3. Supramolecular morphological transition.**
**a**. Schematic illustration of an experimental setup for supramolecular morphological transition.

Page 23, line 624:
**f**. A mechanism of the supramolecular morphological transition.

Page 25, line 632:
**Figure 5. Modulation of the supramolecular morphological transition by low temperature pulses.**

## *Response to Reviewer 2*

**Reviewer 2's comments:**
All my concerns have been reasonably addressed.

**Reply:**
We deeply appreciate your careful review of our manuscript.

## *Response to Reviewer 3*

**Reviewer 3's comments:**
the authors have thoroughly addressed the points raised by the referees, and I'm now happy to recommend the manuscript for publication. I'd like to congratulate the authors on these exciting results.

**Reply:**
We are sincerely grateful for your kind words and your strong recommendation for publication.