nature portfolio

Peer Review File

Acoustically Shaped DNA-programmable Materials

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Referee report for “Acoustically Shaped DNA-programmable Materials” by Arnon et al.

The authors describe a method to assemble DNA origami crystals steered by standing acoustic waves. The combination of using DNA complementarity for local assembly, combined with acoustic steering for the more global arrangement is elegant. Overall, I found it a timely and interesting report. Nonetheless, I believe there are some aspects that should be clarified or improved, outlined below.

- Some of the fonts in the figures are very small. In particular e.g. the scale bars in the insets of Figure 1c and d are hard to read. Use more uniform and larger fonts?

- Figure 3: While it is good to show the individual points for edge length vs. acoustic driving parameter tau, superimposing a box plot or points and error both the mean and std would be very helpful.

- Page 10, line 236: Are there X-ray data also for the crystals assembled without acoustic waves? How different are they from the data for crystals assembled under acoustic guidance?

- The data shown demonstrates crystals in linear stripes. It might be interesting to discuss/speculated about the possibilities –and challenges- of creating more complex patterns?

Minor points:

- Page 10, line 233: “breaking” instead of “broking”?

Reviewer #2 (Remarks to the Author):

In this study, the authors have demonstrated that capacity of acoustic fields with specific spatio-temporal characteristics canto effectively drive the formation of DNA-assembled materials at the macroscale. By combiningThrough the integration of DNA-guided assembly with acoustically driven processes, they researchers have successfullyeffectively control dictated the morphology of DNA-origami-based crystal lattices across a wide range of scales ranging, from tens of microns to millimeters. TheWhile the method presents novelty, while, the observed phenomenon does not strongly support align with the central theme. Consequently, the current version of this, rendering the current manuscript is deemed unsuitable for publication in Nature Communications.
Several questions arise from the presented findings:

1. The experiments, as depicted in the accompanying video and figures, suggest that thea correlation between crystal growth of crystal edges correlates with, DNA density, and temperature. It appears that , with higher DNA density leading to large DNA origami-based lattice structures, regardless of acoustic assistance, can lead to the formation of larger DNA origami-based crystal lattices. The role of aAcoustic waves seems primarily to enhance DNA density in specific regions, rather than facilitating the creation of creating new shapes or origami structures. Thus, it is , challenging to categorize this as ahe genuinely novelty of this acoustic assembly method.
2. In Figure 3, the largest edge length occursit is observed that at τ=20 and n=140, the largest edge length occurs. Could the authors provide an explanation for explain why this specific condition, as opposed to other ones, yields the largest edge length compared to other conditions?
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4.While t The authors mention the ability of their method to obtain millimeter-sized DNA crystals. However, , Figure 4e depicts a long chain of DNA crystals, which does not fulfill the millimeter-sized e criteria of millimeter-sized DNA crystals.
5. If provided withGiven sufficient DNA origami units and longer exposure time under similar temperature increase rates, would the size of DNA crystals formed without acoustic assistance be comparable to those formed with itacoustic assistance?
6. Clarify hHow was the temperature increases were controlled during the experiments, and is. Additionally, address whether there is a possibility that of the interdigital transducer (IDT) contributedcontributing to localized heating of the tested region during long-term tests.?
7. Lastly, the quality of figures in the manuscript appears subpar. It is recommended toneeds improvement enhance the plot quality of the figures for better clarity and presentation.

Reviewer #3 (Remarks to the Author):

In this paper, the authors demonstrate the use of surface acoustic waves to support the assembly of preformed DNA crystals into larger crystals. The waves lead to an accumulation of the structures at the nodes. In combination with a heating effect and material transport, small crystals grow into larger ones and fuse. The process is modeled by a modified nucleation and growth process. This is an interesting and unconventional concept, which could potentially be useful to generate large-scale DNA-based materials.

Specific comments/suggestions:

- Even though the technique is interesting, the resulting structures are somewhat disappointing. What's the use of linear arrangements of DNA microcrystals? Is there a more concrete application idea? Could one shape other more complex structures, potentially multilayered, branched, etc.? At this point, it seems a bit too much to claim that this is a “powerful means to drive the formation of

DNA-assembled materials" (as in the Conclusions on p.11).
- The overall effect is modest. There is an increase in mean edge length, but also a broadening of the distribution – this may be desirable, or not.
- Could one also start with the DNA structures themselves and assemble the crystals in situ?
- Where does the "acoustic contrast" mentioned come from? Is the wavelength of the wave compared to the system's dimensions important? What would happen, e.g., for smaller wavelengths that are closer to the size of the crystals – maybe they would also fuse laterally at one point and create a "sheet"?
- How well can one retrieve the produced material from the surface? E.g., could one take off one of the elongated structures without breaking it?
- Why is the heating effect by the wave "local"?
- In Fig. 1a, indicate the "vertex-driven sixfold hybridization" better that is mentioned in the text.
- Is the data in Fig. 3 (tau = 20 and no pulse) the same as in Fig. 4a?
- There are many typos in the paper and it should be checked for language, e.g.,
p. 2, " ... the six vertices, form a cubic, which Wulff shape is a cube"
→ " ... the six vertices form a cubic lattice, whose Wulff shape is a cube"
p. 12, "tempting" → "templating", etc."

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

Reviewer #1: Referee report for "Acoustically Shaped DNA-programmable Materials" by Arnon et al.

The authors describe a method to assemble DNA origami crystals steered by standing acoustic waves. The combination of using DNA complementarity for local assembly, combined with acoustic steering for the more global arrangement is elegant. Overall, I found it a timely and interesting report. Nonetheless, I believe there are some aspects that should be clarified or improved, outlined below.

Response: We thank the reviewer for the positive and encouraging assessment of our work and for constructive comments and questions that helped us to improve the manuscript. We provide below the responses to the specific comments and the modifications made in the manuscript.

Reviewer #1: - Some of the fonts in the figures are very small. In particular e.g. the scale bars in the insets of Figure 1c and d are hard to read. Use more uniform and larger fonts?

Response: We thank the reviewer for bringing this issue to our attention. The text, fonts and style uniformity were updated to ensure better readability and improved appearance. Also, all figures were re-rendered at 300 dpi for clarity.

Reviewer #1: - Figure 3: While it is good to show the individual points for edge length vs. acoustic driving parameter tau, superimposing a box plot or points and error both the mean and std would be very helpful.

Response: We agree with the referee that box-plot contain key parameters that are important for assessing the results. Although the median, Q1 and Q3 are presented in Figure 4b, following the reviewer's suggestion, we overlayed a box-plot showing the median, Q1, Q3 and 1.5 IQR 'whiskers' to help with the interpretation of Figure 3.

Reviewer #1: - Page 10, line 236: Are there X-ray data also for the crystals assembled without acoustic waves? How different are they from the data for crystals assembled under acoustic guidance?

Response: We thank the reviewer for bringing the important point to our attention. We agree that it is important in the main text to show the x-ray scattering pattern for sample assembled without acoustic filed. We now added a measured structured factor for a sample that was not treated with acoustic waves in Figure 4f. We stress in the text that x-ray scattering patterns are very similar between the modeled structure factor, and measured for both acoustically treated and not treated

samples. This indicates that the acoustic field has an effect on the macroscale morphology only, while nanoscale structure remains the same.

Reviewer #1: - The data shown demonstrates crystals in linear stripes. It might be interesting to discuss/speculated about the possibilities –and challenges- of creating more complex patterns?

Response: We thank the reviewer for allowing us to elaborate on this topic. Indeed, the used interdigital transducers (IDT) geometry can be expended into more complex designs, which can result in different standing waves patterns and that will allow achieving the macroscale morphologies with other shapes. Moreover, recent advancements in controlling acoustic fields offer complex 3D patterning through acoustic holography. We believe that our approach can be potentially combined with both these developments, and our future studies will explore their use. We also hope that our study will motivate researchers to further explore acoustic fields for controlling macroscale formation for different types of nano-assembly process. We added to the "Conclusions" section a discussion regarding this point and the section now reads as follows (Page 11, line 261):

"The macroscale morphology of crystals can be potentially expanded to other geometries, beyond linear structures shown in our work. This can be achieved by changing the boundary conditions of the sample, or by changing the arrangement and geometry of the transducers. For example, complex two-dimensional patterns can be produced, much like Chladni plates[41]. More broadly, acoustic holography can be potentially employed to form complexly designed patterns of self-assembled materials[42,43]."

The corresponding citations are:

41. Kopitca, A., Latifi, K. & Zhou, Q. Programmable assembly of particles on a Chladni plate. *Sci. Adv.* **7**, 7716–7738 (2021).

42. Melde, K., Mark, A. G., Qiu, T. & Fischer, P. Holograms for acoustics. *Nature* **537**, 518–522 (2016).

43. Raymond, S. J. *et al.* A deep learning approach for designed diffraction-based acoustic patterning in microchannels. *Sci. Rep.* **10**, 8745 (2020).

Reviewer #1: Minor points:

- Page 10, line 233: "breaking" instead of "broking"?

Response: We thank the reviewer for attention to details. This typo and others were fixed accordingly.

Reviewer #2 (Remarks to the Author):

Reviewer #2: In this study, the authors have demonstrated that capacity of acoustic fields with specific spatio-temporal characteristics canto effectively drive the formation of DNA-assembled materials at the macroscale. By combiningThrough the integration of DNA-guided assembly with acoustically driven processes, they researchers have successfullyeffectively control dictated the morphology of DNA-origami-based crystal lattices across a wide range of scales ranging, from tens of microns to millimeters. TheWhile the method presents novelty, while, the observed phenomenon does not strongly support align with the central theme. Consequently, the current version of this, rendering the current manuscript is deemed unsuitable for publication in Nature Communications.

Response: We thank the reviewer for the time and effort invested into assessing our manuscript and for the comments and ideas that we believe will improve the work and its readability. We respectfully disagree with the statement ”While the method presents novelty, while, the observed phenomenon does not strongly support align with the central theme”. The majors two themes of the work are focused on using an acoustic field for controlling macroscale morphology and revealing the effect of the field on DNA-based assembly process. Both aims were achieved by demonstrating mm-long wire-type self-assembled structures that sculptured by the acoustic field, and by uncovering the impact of temporal acoustic pulse profiles on crystal growth. We provide below more specific replies, as pertaining to these and other comments.

Reviewer #2: Several questions arise from the presented findings:
1. The experiments, as depicted in the accompanying video and figures, suggest that thea correlation between crystal growth of crystal edges correlates with, DNA density, and temperature. It appears that , with higher DNA density leading to large DNA origami-based lattice structures, regardless of acoustic assistance, can lead to the formation of larger DNA origami-based crystal lattices. The role of aAcoustic waves seems primarily to enhance DNA density in specific regions, rather than facilitating the creation of creating new shapes or origami structures. Thus, it is , challenging to categorize this as ahe genuinely novelty of this acoustic assembly method.

Response: We thank the reviewer for allowing us to elaborate on this key point. First, we would like to point out that according to our calculation, in the power regime of the acoustic field used in our experiments, individual frames are not affected by the field (Figure S5). Thus, there is no concentration increase of monomers. Secondly, even if there was a concentration increase due to the acoustic field, this would not explain why a specific pulsing profile ($\tau = 20$) is more efficient for growth than more frequent pulsing or continuous stimulation. Hence, we would like to stress that the observed phenomena of the effect of acoustic stimulation on the self-assembly of crystals from monomers cannot be reduced to the consideration of concentration only. This phenomenon is not trivial. Indeed, as can be seen in our data (Figure 3, Figure 4 and Figure S6-S18) the crystals growth and its eventual size have a non-monotonic (“resonant”) dependency on τ. If it was only a

matter of material density, then with smaller τ (more frequent pulsing) the crystal size would increase. However, this is not the case, as the maximum crystal size value is at τ = 20, and crystal size decrease as τ decreases. Therefore, pages 8-11 of the manuscript are dedicated to explaining the phenomenon, which was unexpected and discovered in our experiments. Our detailed analysis of data and theoretical modeling allowed reconciling the observation with a mechanistic understanding of this effect. We believe that the discovery of this phenomenon is important from a fundamental perspective for understanding the role of equilibrium and field-induced non-equilibrium effects on the assembly process. To summarize our hypothesis, the growth of crystals under the acoustic waves is subjected to two new effects: (1) Local heating at the active region, and (2) increased influx of small clusters of crystalline nuclei into the active region from adjacent areas in the capillary, increasing effective local concentration. As τ decreases (more frequent pulses), the two effects become more pronounced, which can explain the observed non-monotonic behavior of τ vs. crystal size. To emphasize this point in the manuscript, we have revised the discussion to read as follows (Page 12, line 266):

"Moreover, the acoustic field might enhance the formation of crystals within a certain pulse regime due to the combination of the two new effects caused by the acoustic waves (local heating and influx of nuclei). Our experimental observations are supported by a computational model that incorporated nucleation dynamics, diffusion-limited growth, and the effects of acoustic driving."

Reviewer #2: 2. In Figure 3, the largest edge length occursit is observed that at τ=20 and n=140, the largest edge length occurs. Could the authors provide an explanation for explain why this specific condition, as opposed to other ones, yields the largest edge length compared to other conditions?

Response: We appreciate the reviewer giving us the opportunity to expand on this crucial point. Figure 3 shows the empirical data collected from microscopy images, and indeed for τ = 20 the largest crystal sizes can be observed. We then take pages 8-11, including Figure 4, to explain the non-monotonic dependency of crystal edge length vs τ. The physics-based computational model developed in this work takes the two new effects caused by the acoustic waves into account and addresses the data well. We have revised the conclusion to reiterate this point, which now reads (page 12, line 262) as shown in the reply to the previous comment

Reviewer #2: 3. TThe number density of crystalsvariations in crystal density (n) varies across different conditions in (Figure 3). How can fair comparisons be made between raises questions regarding whether comparisons between unequal densit different conditions with unequal crystal number densitiesies are fair to make?

Response: We thank the reviewer for the question since it is central to understanding the data. The number 'n' below each sample column is the number of crystals measured for that specific sample.

It has nothing to do with the density of crystals or with the amount of DNA in the sample. To avoid a confusion, we have revised the figure legend to explain this point accordingly.

Reviewer #2: 4.While t The authors mention the ability of their method to obtain millimeter-sized DNA crystals. However, , Figure 4e depicts a long chain of DNA crystals, which does not fulfill the millimeter-sized e criteria of millimeter-sized DNA crystals.

Response: We understand that important information the reviewer might not have found, although it was presented in the Figure 4. Indeed, the polycrystalline crystal shown in panel 4e is at the millimetric scale, - it is about 2 mm long. This crystal is formed by fusing single crystals into mm-scale morphology which shape is defined by acoustic field geometry. To prove that this is one long structure, we point a reader's attention to the fact that this structure 'hops' between nodes (highlighted in red) without breaking into two separate sub-structures. This aspect is also discussed in the text. For scale comparison, - the capillary width in the image (from left to right) is 1 mm. To underline this issue, we have revised the figure legend to explicitly indicate the capillary width. In addition, we have included two more videos of the process, Vid S6 and Vid S7, both showing the assembly of crystals at different acoustic conditions with end products of linear structures of above 1mm (the entire frame and more).

Reviewer #2: 5. If provided withGiven sufficient DNA origami units and longer exposure time under similar temperature increase rates, would the size of DNA crystals formed without acoustic assistance be comparable to those formed with itacoustic assistance?

Response: We thank the reviewer for this excellent question. While DNA concentration affects the resulting crystals' size in a functionally monotonic way, the dependency of crystal size on concentration will not produce such a non-monotonic behavior as observed with our acoustic experiment. Elevated DNA concentration will increase both nucleation and growth rates, while the acoustic standing waves increase growth rate and suppress nucleation, which is quite a non-trivial effect. Hence, the dependency on these two processes has a different effect on the crystal size of the population, which is presented in in the manuscript.

Reviewer #2: 6. Clarify hHow was the temperature increases were controlled during the experiments, and is. Additionally, address whether there is a possibility that of the interdigital transducer (IDT) contributedcontributing to localized heating of the tested region during long-term tests.?

Response: We thank the reviewer for the opportunity to further explain this point. The temperature was controlled using a Linkam LTS420 Thermal Stage. The Linkam LTS420 Thermal Stage is a thermal cell that can be mounted on an optical microscope and is controlled by computer software with thermal stability of ~0.01 ºC at a range of from room temperature to 50 ºC in our setup. The sample was placed on the acoustic device, which was then placed inside the Linkam cell and the cell was closed to ensure thermal stability. The SI was revised to detail this setup.

Figure S1 shows the local temperature measured in the active region between the IDTs for different τ values. This gives us important insight into the excess heating due to the acoustic wave pulsing without the use of a thermal stage that can mitigate the heating effect. The temperature was measured in the active region with thermal paste to conduct and measure the heat properly. We have revised the legend of Figure S1 to convey this point, and it now reads as follows:

"**Figure S1.** Temperature change induced by the acoustic waves in the active region. τ is defined as the period divided by the pulse length. τ=1 means constant waves are applied. Waves were applied after 60 seconds at room temperature (25.5 °C) without the use of active temperature control. τ values in the experiments conducted were between 10 – 100. Thermal paste was used to ensure proper temperature measurement of the active region."

The temperatures in Figure S1 were measured for 30 minutes, and it is clear that there is no significant change in temperature beyond the 10^{th} minute of the measurement.

In our experiments, the lowest τ value that was 10 to avoid excessive heating.

Reviewer #2: 7. Lastly, the quality of figures in the manuscript appears subpar. It is recommended toneeds improvement enhance the plot quality of the figures for better clarity and presentation

Response: We thank the reviewer for this comment. We have increased the text size on all figures, unified the style of the text and re-rendered all figures at 300 dpi; we believe the changes improve readability and clarity of the figures.

Reviewer #3 (Remarks to the Author):

Reviewer #3: In this paper, the authors demonstrate the use of surface acoustic waves to support the assembly of preformed DNA crystals into larger crystals. The waves lead to an accumulation of the structures at the nodes. In combination with a heating effect and material transport, small crystals grow into larger ones and fuse. The process is modeled by a modified nucleation and growth process. This is an interesting and unconventional concept, which could potentially be useful to generate large-scale DNA-based materials.

Response: We thank the reviewer for providing positive assessment of our work and its innovative nature. We are also grateful for the useful suggestions below, which we addressed in the revised manuscript. These changes greatly contributed to an improving the manuscript.

Reviewer #3: Specific comments/suggestions:

- Even though the technique is interesting, the resulting structures are somewhat disappointing. What's the use of linear arrangements of DNA microcrystals? Is there a more concrete application idea? Could one shape other more complex structures, potentially multilayered, branched, etc.? At this point, it seems a bit too much to claim that this is a "powerful means to drive the formation of DNA-assembled materials" (as in the Conclusions on p.11).

Response: We thank the reviewer for this important comment. We envision that this technique could potentially lead to implementation into optical devices, such as, for example, waveguides, optical metamaterials or Bragg reflectors, where both nanoscale order and macroscale morphology is important. While the specific applications are beyond the scope of this study, we believe that this work brings us closer to such realization of such devices and materials and it will be useful for researchers working at the interface of nanomaterial assembly and optical applications. The focus of our study is on understanding the basic science and the underlying mechanism for the observed phenomena, including both formation of macroscale morphology and crystal growth under the acoustic stimulation. We would like to point out that this approach can be complemented with other techniques developed in our labs and other labs to functionalize and template the formed structures post-assembly. This opens the possibility to assemble and then process nanostructures by controlling not only the macroscale morphology but also inorganic material composition; this should find many useful applications. For specific examples of such processes, please see our previous work, where we used silicon and templating for converting DNA frameworks into a variety of materials (Michelson et al., Science 2022 and Michelson et *al.*, *Sci. Adv.* 2024, both of which are cited in the manuscript in the appropriate locations).

We fully agree with the reviewer that the concept of more complex structures is alluring and interesting. We also stress in the revised manuscript that by changing the transducer geometry and employing acoustic holography, more complex and even arbitrary "sculpturing" of assembled morphologies is feasible using the principles established in our study. Thus, we have revised the conclusions section, which now reads as follows (page 11, line 259):

"This study shows that the acoustic field can drive the assembly of macroscale morphologies from preformed crystals and monomers under annealing conditions. The macroscale morphology of crystals can be potentially expanded to other geometries, beyond linear structures shown in our work. This can be achieved by changing the boundary conditions of the sample, or by changing the arrangement and geometry of the transducers. For example, complex two-dimensional patterns can be produced, much like Chladni plates[41]. More broadly, acoustic holography can be potentially employed to form complexly designed patterns of self-assembled materials[42,43]."

The corresponding citations are:

41. Kopitca, A., Latifi, K. & Zhou, Q. Programmable assembly of particles on a Chladni plate. *Sci. Adv.* **7**, 7716–7738 (2021).

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43. Raymond, S. J. *et al.* A deep learning approach for designed diffraction-based acoustic patterning in microchannels. *Sci. Rep.* **10**, 8745 (2020).

We have also revised the conclusion to make the stated claim more moderate, and replaced the word “powerful” with the word “effective”.

Reviewer #3: - The overall effect is modest. There is an increase in mean edge length, but also a broadening of the distribution – this may be desirable, or not.

Response: We thank the reviewer for the invitation to elaborate on this topic. To clarify, there are two main effects discussed in this work. The first one is the macroscale organization of the crystals into an elongated linear morphology. This effect is dramatic in its power to form large-scale structures, as directed by the acoustic field, in comparison to tens microns crystals that are dispersed in solution with no acoustic driving. As we mentioned in reply to the previous comment, using more elaborative geometry of transducers and acoustic holography, we can envision an arbitrary control over the desired macroscale morphology with maintained nanoscale organization. The crystals fuse into a single entity, which can promote the use of this technique in a manner that is not applicable without the acoustic organization. The second effect is the effect on crystal size. While we agree that this effect is rather modest (*i.e.*, ~ +75% in mean crystal size for the strongest effect), this phenomenon was also unexpected. The observation of larger crystals was not part of our design scheme leading us to this work. We believe that understanding this mechanism thoroughly will allow future works to take advantage of this phenomenon and harness it to our advantage, as it has important fundamental value.

Reviewer #3: - Could one also start with the DNA structures themselves and assemble the crystals in situ?

Response: We thank the reviewer for this question, which in part motivated this study. In fact, we have done exactly what the reviewer suggests. There are two level of answer, depending on the initial starting point of DNA structures, origami frame monomer, and lattice frameworks formed by these monomers. The manuscript described these two approaches. The first approach is aligning pre-formed crystals (domains of frameworks) and fusing them together. The second approach is to form the crystals, starting from monomeric DNA frames, while the acoustic waves stimulate the sample. If we understand the comment correctly, the second approach is what the reviewer suggests – starting from the DNA frames as monomers and assembling the crystals in situ during the active acoustic excitation.

To make this point clearer, we have changed the titles of the sections to read as follows:

First approach (page 5, line 87): “Acoustic organization of pre-formed crystals”

Second approach (page 7, line 137): “Crystallization under acoustic stimulation”

Reviewer #3: - Where does the “acoustic contrast” mentioned come from? Is the wavelength of the wave compared to the system's dimensions important? What would happen, e.g., for smaller

wavelengths that are closer to the size of the crystals – maybe they would also fuse laterally at one point and create a "sheet"?

Response: We are grateful to the reviewer for enabling us to provide further details on this important aspect. The section "Acoustic contrast factor" in the Supplementary Information provides information for acoustic contrast considerations. We have revised the main text to include a pointer to the SI where the acoustic contrast is first introduced.

The acoustic wavelength employed in our work (order of 100 μm determined by the used transducer) was part of the experimental design. We considered having a wavelength larger than typical crystallites to force them into morphologies larger than their sizes and structures that cannot be easily formed based on equilibrium assembly. On the other hand, the wavelength should be fine enough for shaping morphological features. Thus, we decided to proceed with wavelength of one order of magnitude larger than the crystal size (order of 10 μm). In this case, crystals can be experiencing sufficient force to be pushed towards the nodes without being too close to the antinode or to the neighboring node. Changing the transducer and container architecture and the wavelength can change the distance between the nodes. However, in between two nodes, there will always be an antinode, preventing 'sheets' from being formed. For the wavelength smaller than a crystal size, it is possible that at the large intensities of the field and for crystal being in both nodes and antinodes zones, a crystal might break. This is an interesting effect to consider for the future studies, but it requires to have a different type of transducers and to redesign the other related part of the setup.

That said, there is an interesting discussion to be made about more complex IDT design, which can give rise to other macroscale morphologies. In order to emphasize, the "Conclusions" section was revised and now reads as follows (Page 11, line 261):

"The macroscale morphology of crystals can be potentially expanded to other geometries, beyond linear structures shown in our work. This can be achieved by changing the boundary conditions of the sample, or by changing the arrangement and geometry of the transducers. For example, complex two-dimensional patterns can be produced, much like Chladni plates[41]. More broadly, acoustic holography can be potentially employed to form complexly designed patterns of self-assembled materials[42,43]."

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43. Raymond, S. J. *et al.* A deep learning approach for designed diffraction-based acoustic patterning in microchannels. *Sci. Rep.* **10**, 8745 (2020).

Reviewer #3: - How well can one retrieve the produced material from the surface? E.g., could one take off one of the elongated structures without breaking it?

Response: We thank the reviewer for this question, which might be important for the future use of our methodology. The extraction of the produced macroscale morphology can be achieved by breaking the wax seal of the capillary and then using a pipette to push the liquid out of the capillary. The SI was revised to include this important detail and now reads as follows (SI page 4, line 81):

"The end product can be extracted from the capillary by breaking the wax seal and pushing the liquid out using a pipette with a gel loading tip."

Potentially, more elaborative solutions based on the integration with microfluidics can be developed.

Reviewer #3: - Why is the heating effect by the wave "local"?

Response: We thank the reviewer for bringing this point since it was not clearly discussed in the manuscript. The measured temperature of the sample under acoustic filed stimulation, as given in Figure S1, is 'local temperature' (i.e. a temperature of the active region where acoustic waves are present) since it was measured by a thermocouple probe placed at the acoustically active region with thermal paste between the probe and surface. Thus, the term local temperature refers the temperature at the active region as oppose to other regions in the capillary where the acoustic waves are not present. We added a related clarification to the revised text. Our experiments were conducted in a regime with a moderate heating effect (τ = 10 to 100). We have also revised the legend of Figure S1 to convey this point, and it now reads as follows:

"**Figure S1.** Temperature change induced by the acoustic waves in the active region. τ is defined as the period divided by the pulse length. τ=1 means constant waves are applied. Waves were applied after 60 seconds at room temperature (25.5 °C) without the use of active temperature control. τ values in the experiments conducted were between 10 – 100. Thermal paste was used to ensure proper temperature measurement of the active region."

Reviewer #3: - In Fig. 1a, indicate the "vertex-driven sixfold hybridization" better that is mentioned in the text.

Response: We thank the reviewer for this suggestion. We have revised Figure 1a to specifically state that the self-assembly is driven by a sixfold vertex hybridization. This is now stated both in the main text and in the figure itself.

Reviewer #3: - Is the data in Fig. 3 (tau = 20 and no pulse) the same as in Fig. 4a?

Response: We thank the reviewer for bringing this to our attention. The histogram data of crystal size shown in Figure 4a is shown in a different representation in Figure 3. We have revised the legend of Figure 4a, which now reads as follows (page 11, line 242):

"**a,** Histogram of crystal size distribution with no acoustic waves (blue) and with τ = 20 (red) for thermal ramp down of '*fast'* 0.03 °C/min (some of the data is shown in Fig. 3 with different representation)."

Reviewer #3: - There are many typos in the paper and it should be checked for language, e.g., p. 2, " ... the six vertices, form a cubic, which Wulff shape is a cube"
→ " ... the six vertices form a cubic lattice, whose Wulff shape is a cube"
p. 12, "tempting" → "templating", etc."

Response: We thank the reviewer for the careful reading of our manuscript and all suggestions and comments that helped us to improve the manuscript. We also corrected these and other typos.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed my previously raised point in the revision and I support publication of the manuscript at this point.

Reviewer #2 (Remarks to the Author):

The author did a great job responding to the reviewers' comments. The article can be accepted as it is.

Reviewer #3 (Remarks to the Author):

The authors have appropriately addressed all of this reviewer's comments.
It is recommended to accept the paper for publication in Nature Communications.