**Rebuttal Letter**

Our answers to editorial and referees’ comments are listed under each question.

**Editorial comments:**  
Changes to be made by the Author(s):  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Yes, we have thoroughly proofread and revise our manuscript.

2. Please submit the figures as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300dpi.

Yes, we submit the figures as a .tif and ensure the image is 1920 pixels x 1080 pixels or 300dpi.

3. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.

Yes, we check all references following JoVE’s rule.

4. Please include volume and issue numbers for all references.  
Yes, we do.

5. Please define all abbreviations before use.

Yes, we do.

6. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

Yes, we do.

7. Unfortunately, there are a few sections of the manuscript that show overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please see lines: 45-56.

Thanks, we have rewritten these sentences.

8. Figure 3: Please define the units of the ladder: nt, bp? It would help to use panel labels as well (A, B, etc.).

Thanks, we have labeled the ladder with the unit of bp in Fig. 3.

9. Is there a Z scale for the AFM images?  
Thanks, we have added the Z-scale to AFM images.

10. Please provide the sequences in a separate table from the Materials Table.  
Thanks, we have provided the sequences in a separate Table of DNA Sequences.

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.  
Thanks, we have rewritten parts of the protocol.

12. 1.4: How is the T4 ligase reaction done? How is the 5’ phosphorylation done?  
Thanks, the 5’-phosphorylated oligos can be directly ordered from commercial companies. We added the T4 ligase reaction in lines of 101-107, or in protocol steps of 1.5-1.6.

13. 1.9: What percentage PAGE?  
Thanks, we added 10% PAGE for both denaturing and native PAGE.

14. What are the size of the gel plates? V/cm is a more useful parameter.  
Thanks, we added the size of both PAGE and agrose gels in lines 131 and 247 respectively.

15. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.  
For example: GelRed, etc.  
Thanks, we deleted all trademark symbols (™), registered symbols (®), and company names before an instrument or reagent, for example, GelRed and Bruker FastScan AFM.

16. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.  
Yes, we do.

17. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.  
Yes, we do.

18. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:  
a) Critical steps within the protocol  
b) Any modifications and troubleshooting of the technique  
c) Any limitations of the technique  
d) The significance with respect to existing methods  
e) Any future applications of the technique  
Thanks, we have revised the Discussion.  
  
**Reviewers' comments:**  
  
Reviewer #1:  
  
Manuscript Summary:  
This manuscript describes detailed protocols on DNA nanoconstructions with small circular DNA, including small circular DNA preparation and purification, DNA nanostructure assembly and characterization. This manuscript is based on the series of works from the group of Dr. Shoujun Xiao, the senior author. It is a unique and elegant branch in DNA nanotechnology. This referee recommend for publication as it is.  
Many thanks.  
  
Reviewer #2:  
  
Manuscript Summary:  
The manuscript of "Stable DNA Motifs, 1D and 2D Nanostructures Constructed from Small Circular DNA Molecules" from Xin Guo, Xue-Mei Wang, and Shou-Jun Xiao, as a visualized experimental protocol for JoVE, is well presented for the detail protocol. The following detailed protocols are described: 1) preparation and purification of small circular oligonucleotides, 2) annealing of stable circular tiles, followed by native PAGE analysis, 3) assembling of infinite 1D nanowires, nanorings, and nanospirals, infinite 2D lattices of nanotubes and nanoribbons, and finite 2D rectangles, followed by AFM imaging. This method for building DNA 1D and 2D nanostructures is simple, solid, and affordable for most labs. It is worth of publication after minor revisions listed below.  
Appreciate.

Minor Concerns:  
1 In the "Introduction" part, when a specific tile or lattice name appears, for easy following, its corresponding figure should be noted.

Thanks, we added a sentence in a bracket “(please refer to Figures 3-5 for the schematic drawings and images of the above five kinds of DNA nanostructures)” in lines of 62-63.

2 The composition of TE buffer should be described when TE buffer first appears. Thus the TE buffer composition in 1.4 should move to 1.2.

Thanks, we revised it.

3 Correct the typos of "Table of Materilas" to "Table of Materials" in figure captions of Figure 3 to 5.

Thanks, we revised them.

4 Maybe due to format, the page 24, 26, 28 ,32, 41, 42, 44 look strange.

Thanks.

5, for the figure 2, the image looks quite blurry. Maybe the inverted images will look better on the contrast.

Thanks for pointing out the imperfect photo of Figure 2, we try our best for Fig. 2.