October 31st, 2012

To

The Editorial Board

Journal of Visualized Experiments (JoVE)

**Re: Author responses to reviewers comments**

**Manuscript titled “Designing a bio-responsive robot from DNA origami”**

Dear Editorial Board Members,

We would first like to apologize for the delay in submitting our revised version of this paper to the Journal of Visualized Experiments. We hope that no inconvenience was caused to the editorial process because of this.

We have carefully read the comments made by our 4 reviewers, and we must admit that the ultimate selection of reviewers by the Journal was superb. Clearly made by experts wishing to assist us in this task, these comments resulted in significant improvements to the manuscript, and we are deeply grateful to both the reviewers and the journal for giving us this opportunity to learn and improve.

Below is a point-by-point response to the reviewers’ comments. We hope you will find the manuscript suitable for publication, and we would welcome as usual any further comment or request for clarification made by the Journal or any of the reviewers.

Best Regards,

Ido Bachelet, Ph.D.

Corresponding Author

**Author responses to reviewers’ comments**

Below is a list of responses to the reviewers’ comments on our manuscript titled “Designing a bio-responsive robot from DNA origami”.

In their papers, Douglas and Dietz already put forward the foundations of DNA origami design in great detail. Our purpose in the current paper was twofold: a) to provide a highly simplified guide enabling the inexperienced user to rapidly obtain only the technical knowledge, omitting for the time being the thorough theoretical basis, needed to design a DNA origami object; b) to put the emphasis on designing a functional DNA origami device, that responds to biological inputs, rather than a “regular” shape.

As a major conclusion from the reviewers’ comments, we have drastically revised the manuscript around two points:

1. Using caDNAno 2.0 from within Maya 2012
2. Demonstrating the design process of a robotic device similar to the one described by Douglas & Bachelet (Science, 2012), as a device already tested and proven functional

Nevertheless we would like to clarify why a toy example designed by caDNAno 1.0 was initially chosen for this paper.

**On preferring caDNAno 1.0 over caDNAno 2.0 as the interface of choice for untrained beginners**

This point recurred in all the reviewers’ comments. This point relates to the use of the older caDNAno 1.0 rather than the newer version, caDNAno 2.0, as the interface of choice for beginners. Indeed, caDNAno 2.0 has several features, which constitute significant advancement over caDNAno 1.0.

Nevertheless, our initial choice of caDNAno 1.0 derived from the following points:

1. The caDNAno 1.0 workflow is more ‘structured’, less flexible and less intuitive (e.g. it does not include automatic crossover generation during initial scaffold drawing, has separate tools for adding staples and erasing them, etc.). As this paper is intended to give the general reader, particularly readers from outside the field of DNA origami with zero training in DNA design, a first hands-on experience in this mission, we felt that these could actually become advantages, enabling the user to carry out each step separately and receive only the relevant explanation for this step. Indeed, using caDNAno 2.0 one might generate the same DNA origami structure significantly faster, but this is not an advantage for a beginner, but rather for the experienced user.
2. The excellent 3D capabilities of Autodesk Maya can be used to generate a 3D model from a .json file made in both caDNAno 1.0 and 2.0. While caDNAno 2.0 can be operated from within Maya, the caDNAno 1.0 3D viewer is primitive; however, operating caDNAno 2.0 from Maya requires the user to learn at least to some extent the relatively complicated interface of this software, and users without experience in 3D modeling might find this somewhat intimidating.
3. Very clear and didactic video tutorials are available for caDNAno 1.0 but not for caDNAno 2.0.
4. Finally, we collected feedback from untrained users, including high-school students and DIY-bio hobbyists, and these convinced us that caDNAno 1.0 would be the better choice as a beginners’ interface for designing DNA origami shapes.

However, after careful consideration of the reviewers’ comments we became convinced that re-writing the article to focus on caDNAno 2.0 rather than 1.0 would serve our purpose better.

**Limitations of the methods should be discussed and the authors should provide a better explanation in each figure legend.**

We added a paragraph describing the technical difficulties of using DNA origami devices as a therapeutic platform. The legends to figures […] have been rewritten in further detail.

**When discussing the actual folding of the shape, the authors should cite the review article by Bathe's and Deitz's group: "A primer to scaffolded DNA origami" which appeared in Nature Methods last year. This has details on the folding conditions and goes into greater depth on the physical aspects of DNA origami. They should also explicitly cite the caDNAno tutorials on the caDNAno website.**

We thank the reviewers for noting this unfortunate omission. We have added this important paper and properly referenced it.

**The present article and video will be a useful tutorial complement to these existing articles but goes into less depth on the basic principles of DNA origami in the interest of walking the viewer/reader through a full applied example.**

We agree with this remark. However, we would like to clarify that our idea in proposing this protocol to *JoVE* was to deliver to the reader a highly simplified learning experience, based on a “toy example” of a DNA robot. In contrast to the original caDNAno paper by Douglas and colleagues, which demonstrates caDNAno through a short fragment of a 6-helix nanotube, our emphasis here was to walk the reader through the design of a “live” robot with a logic gate. We encourage the reader throughout the article to complement this applied know-how with reading the more detailed and theoretical papers by Douglas and Dietz, which make the foundations for the caDNAno design interface.

As stated above, in writing this paper we collected responses from untrained users, who were given this manuscript and asked to follow the instructions step by step. Our impression was that the manuscript provided a sufficient technical understanding of caDNAno to enable reconstruction of our toy robot in less than 30 minutes after starting from a zero knowledge point, which was our purpose here.

**The screenshots are from the first version of caDNAno, which is now considered a legacy version by some users. They should give links to the v2 and v1 software in the text and explain the differences (which are minor for our purposes here).**

The manuscript was changed accordingly, see p. 1 of this letter.

**In discussion the creation of the basic helix geometry (left panel), the authors could note that for a circular scaffold path, the natural arrangement of helices divides the structure into groups of two helices. Then "internal" crossovers are made within the groups of two and "external" crossovers are made between them. This allows the approximation of many shapes using two-helix building blocks. The authors could explain that this constraint is relaxed for a linear as opposed to circular scaffold.**

We thank the reviewers for this comment. We have added this explanation in the appropriate place.

**Some of the writing could be cleaned up a bit. For instance in the short abstract the phrase "subsequently relayed to a desired effect" could be removed. Likewise the sentence "We are very much hopeful that people will find warm uses that we can think about" in the discussion seems unnecessary. In general making the writing somewhat shorter and tighter could improve the flow but is not necessary.**

We have revised the manuscript to exclude these and similar statements.

**The paper demonstrates the creation of a 'toy' example which roughly approximates the shape from the referenced Science paper. This alternative requires additional steps to generate an appropriately short scaffold strand, and even recommends leaving in 4000+ bases of unwanted scaffold. Did the authors actually make this smaller device? Have functional assays been used to demonstrate it works? Why not just show how to construct the previously published device?**

The manuscript has been changed accordingly, see p. 1 of this letter.

Finally, to answer the reviewer’s important question: we have indeed tested two schemes of folding small origami shapes:

1. Computationally fragmented scaffold. In this method, the shape is designed as usual, a scaffold is chosen, and an .svg file is exported to Adobe Illustrator to view the part of the scaffold sequence that is actually used. This sequence is then split to fragments in Illustrator, making sure splitting is carried out to ensure optimal overlapping between staples and scaffold fragments.
2. A digested ssDNA as a custom scaffold. Here we simply choose a restriction fragment of any available ssDNA, which corresponds with our desired scaffold length, and paste the digested sequence as a custom sequence in caDNAno.

Both of these were used to fold a small, roughly cubic DNA origami object of ~0.6 Kb total length, and were successful in some of the attempts based on gel electrophoresis results.

**It seems the authors are suggesting some preparation steps that might not work very well. Specifically, there are published protocols for generating scaffold, folding, and then purifying a full-size, M13-based origami. But is it actually so easy to purify a 1kb-origmi from a 4kb unused phiX174 scaffold fragment, as the authors suggest? They claim that the fragment is 'irrelevant', but unless they have tested this, it is not obviously true.**

We fully agree with this point, and the manuscript has been revised accordingly.

**Gel electrophoresis, and microscopy (TEM or AFM) are widely used methods for characterization of DNA origami. While the authors present several nice screenshots of caDNAno and CanDo, they do not offer any example experimental assays (with proper controls) to help the reader check whether or not they are successfully following the presented protocols. If the authors could include a gel and some AFM or TEM images of the described shape, it would potentially address my concerns described in #1 and #2.**

Interpreting gel or AFM/TEM images of DNA origami shapes has been described. We felt it would be better to strip the current article from discussing interpretations, for the sake of making it focused entirely on the design aspect, while referring the reader to several of numerous examples of how DNA origami objects should appear on a gel or micrograph.

Still, we fully agree with this point in general, and would like to propose a follow-up paper focusing on both examining the folding by gel and AFM/TEM, and some bioassays testing the function of the device on living cells.

If it were believed that the current article would not be of value without this discussion and data, we would then propose to rewrite it using a device we already built and tested, for which such data exists.

**In Step 1.1: Suggests to download the software from** [**cadnano.org**](http://cadnano.org/)**. When visiting the site, it's possible to easily download version 2 of the software, but what is shown here appears to actually have been downloaded from here:** [**http://cadnano.org/legacy**](http://cadnano.org/legacy)

The manuscript has been revised accordingly, see p. 1 of this letter.

**One of my main concerns is that the article focuses on the use of an older (now obsolete) version of caDNAno which is no longer available for download at the website and has been superseded by a more user-friendly (i.e. now featuring an undo function) version with increased usability and display possibilities (i.e. the integration into Autodesk Maya). Although not entirely different from the version described in this manuscript, the workflow as well as the steps to create a DNA origami structures are somewhat different. I think - although this might mean an extensive change - that the authors should rewrite the affected parts of the manuscript (protocol and figures) with focus on the current version of caDNAno.**

The manuscript has been revised accordingly, see p. 1 of this letter.

**Figure 1a and 1b should be switched. 1a should show the closed state and 1b the open. At the moment this is mixed up.**

The manuscript has been revised drastically and the entire figure layout has changed and re-checked for consistency.

**Page 4, section 1.4: "Note that helices no. 25 and 0 connect ?" At this point in the design process, there is no helix no. 25 yet, neither in the helix side-view (panel 1) nor in the diagram view (panel 2).**

We thank the reviewer for pointing this out. This sentence was included by mistake and was properly removed.

**Page 5, section 3. I think that the mechanism of creating locks for the desired device is not explained well enough. Readers might be confused by the fact that the staple strands designed to lock the device are not connected to anything during the design process. I think it would be important to mention, that this actually binds to the second part of the shell that makes up the complete device (as depicted in the closed state in Figure 1).**

We have included a specific explanation for this potentially confusing part. We also included a reference to Fig. 1 which clarifies the role and context of the gate as a part of the complete device.

**Page 6, section 4.2: It should read "repeat step 4.1" not 2.2.**

This error was properly corrected.

**Point 5.1 needs clarification. Can one use a circular scaffold? If yes, is the start and end point created in this step important?**

A break needs to be inserted into the scaffold strand so a sequence can be assigned to it. In the actual reaction, the scaffold strand (e.g. M13mp18 ssDNA) is in fact circular, and usually does not need to be linearized although this depends on the specific shape folded (see Rothemund, 2006). We agree with this point, and clarified the manuscript accordingly.

**Page 7, section 6.1: Can this assignment be done automatically by leaving the helper scaffold strands on the respective helices in place and assigning a different sequence to these?**

Yes it could; however, because both the gates and the loading strands are important for function, they are not usually automatically assigned. The gates should be specific DNA sequences that respond to the desired cue (e.g. aptamers that bind a target protein, or a DNA restriction site that is cleaved in the presence of the proper nuclease, etc.). Loading sites can be automatically assigned, but these sequences need to hybridize with the strands covalently linked with the cargo molecules; since usually the same strand is used for different devices, the loading sites will have the same sequence and therefore should not be automatically assigned.

**Page 8, section 6.2.1: I would change "biological cue" to "biological input". This should also be changed in the abstract (page 2). Also "profile of cues" should be changed to "sequence of inputs" maybe?**

‘Cue’ has been changed to ‘input’ throughout; ‘profile of cues’ was changed to ‘signature of inputs’ – we hope that this satisfies this requirement and would gladly consider alternatives.

**Page 9, section 8: The part "? that the relevant scaffold fragment makes up less than half of the total material ?" might be obvious for researchers in the field but less intuitive for "newcomers". This part would benefit from a one or two-sentence explanation what this exactly means.**

We thank the reviewer for raising this point, and we have included an appropriate explanation of this heuristic.

**Page 11, description of figure 22: The third sentence should be rephrased for better clarity.**

The sentence was rephrased as requested.

**Page 11, description of figure 23: RMS deflections in nm don't make sense here as there is no scale bar with absolute values. I would delete the "nm" part.**

We agree but preferred to keep the ‘nm’ and manually add a color scale bar for RMS deflections.

**In the discussion (page 12): What do the authors mean with "? scientific devices from DNA origami"**

This has been clarified in the manuscript. Our meaning was that in addition to therapeutic tools, one could design DNA origami research device, e.g. a device that places two proteins in a specific spatial configuration upon receiving a biological input (such that the device is inducible), enabling to record the output resulting from this artificial placement of a protein-protein pair.

**Again: cues should be changed to inputs**

‘Cue’ has been changed to ‘input’ throughout

**"Alternatively, restriction enzyme was already" seems to be incomplete**

This typo was corrected and the sentence is now complete.

**The remote control approach is an interesting aspect. Could this also be achieved by a user-supplied synthetic biological input signal? How would that work in a living organism?**

In fact the interesting possibility of generating remote control interfaces for DNA origami devices based on synthetic biology is a topic of study in our lab. Some of the ideas include programming the device to respond to a remotely inducible input such as a hormone or a neurotransmitter. A more advanced possibility is to use an inducible fragment of DNA as a ‘walk-in’ gate, meaning the devices are deployed gateless, and once the gate is synthetized by a user input, it attaches to the device in the proper position and functionalizes it. We actually added a short discussion on user interfaces with origami devices as this point is closely related to the purpose of this article.

**"We are very much hopeful that people will find warm uses that we can think about". This sentence should be rephrased**

As mentioned above, this sentence has been rephrased and the entire manuscript has been revised throughout for such unnecessary additions.

**Last sentence: "? which can be replaced by any user because the are all compatible" should be rephrased.**

This sentence has been erased.