**Dear Xiaoyan Cao,**

**We would like to thank the editorial staff for their care in reviewing this manuscript. We also appreciate comments from the peer reviewers and thank them for their time. We have addressed all comments. Please see replies in red next to each, below.**

**Best,**

**Markwalter et al.**

**Editorial comments:**  
Changes to be made by the Author(s) regarding the written manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. – We have reviewed the document.  
2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].” – We have Elsevier permission and the document is included in this submission showing such.  
3. Figure 6: Please use capitalized letters for panel labels as in other figures.- We have corrected the figure to have capital letter labels.  
4. Please rephrase the Long Abstract to more clearly state the goal of the protocol.- We added two sentences and slightly rearranged the abstract to clarify the goals.  
5. Please rephrase the Introduction to include a clear statement of the overall goal of this method. – We provided clarification starting at line 130.   
6. Please revise the protocol (e.g., Lines 147-148, 255-271, 326-332, 365-368, etc.) to contain only action items that direct the reader to do something (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.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. - We removed many details from 1.1.1 that did not fit the imperative tense and placed them in Section 1 of the SI. Section 1.5.2 was modified to the imperative, with details provided as a note. The changes at 326-332 were made by combining it as a note to the previous instruction (and later moving it to the discussion as noted below). Portions of the 365 section and others in that vicinity were reworded or moved to the SI. All instructions are now be in the imperative tense.  
7. In the JoVE Protocol format, “Notes” should be concise and used sparingly. They should only be used to provide extraneous details, optional steps, or recommendations that are not critical to a step. Any text that provides details about how to perform a particular step should either be included in the step itself or added as a sub-step. Please consider moving some of the notes about the protocol to the discussion section. – We have reduced extensive notes by moving to the SI, results, or discussion. In some cases, the wording was simplified or removed. In particular, we simplified the note in 1.3.1 and 1.3.4 but have kept it in the protocol because it relates directly to the step. We moved 1.4.7 to the results section. Some notes (e.g. what was originally 1.5.2 and 1.5.3 as submitted) were moved to the discussion section. We believe that these are important to include and so we have not placed them in the SI.   
8. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please move the discussion about the protocol to the Discussion. Some examples:  
1.1.1: Please break up into sub-steps. – We addressed this as noted above.  
1.4.7, 1.5.4, 3.1.6: Please consider moving it to Discussion.- We moved 1.4.7 to the results section where it fit nicely. We have reorganized 1.5.4 into the discussion. We have moved 3.1.6 to a different location and shortened the explanation. However, we want to keep it in the protocol section because it aids in understanding why the mechanical stops are necessary and how to align them properly – this is an important safety consideration to avoid shattered glass. Other changes have been made to other portions of the protocol that we felt needed addressing based on the guidance provided here.

9. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. – We addressed the specific examples below as well as modifications to a number of sections to meet these requirements.

Some examples:  
1.3.7: Please specify centrifugation parameters (force and time). – We added this information in each of the relevant steps.  
1.5.1: Since this step is highlighted for filming, please describe the action in more details so it can be replicated. – We added additional details. However, the instrument analysis will vary depending on if it is supplied by a company or custom built, so we have maintained that as being more vague. It is not central to the protocol’s success.  
3.3.5, etc.: Please specify the type and volume of solvent used in the step. – We added additional details to clarify the cleaning steps.  
Lines 440-441: As this is highlighted for filming, please describe the specific actions. – We added additional details or references to the section that is being replicated here.   
10. Please include single-line spaces between all paragraphs, headings, steps, etc. – This has been implemented.   
11. After you have made all the recommended changes to your protocol (listed above), please re-evaluate your protocol length. There is a 10 page limit for the Protocol. Please revise the protocol section to meet this page limit. – We are under this limit after addressing some of the brevity comments for the notes.  
12. There is a 2.75 page limit for filmable content. 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. – We have modified the highlighted portions based on the changes. We would like to include some videography of Section 4 but do not need to show many details. Just general operation.  
13. 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. – We believe that everything selected fits this criteria.  
14. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted. – We believe the highlights capture all of the important steps.  
15. Discussion: Please mention any limitations of the technique.- We rephrased the beginning of the second discussion paragraph to more clearly indicate that we are addressing the limitations of the technique at that point. The process is constrained by solubility and solvent interactions.  
16. Somewhere in the manuscript, please mention that CAD files for the CIJ and MIVM mixers are available in supplemental files. – This has been added where “procurement” is mentioned in the protocol.   
  
**Reviewers' comments:**  
  
  
  
**Reviewer #1:**  
Manuscript Summary:  
This manuscript describes a versatile and facile preparation of nano emulations that encapsulate therapeutics. The manuscript is well written, with a clear background introduction, easy-to-follow operation protocol and thorough discussion. I recommend publish this manuscript with some minor revisions. Please see my comments below. – We thank the reviewer for their time and care in reviewing.  
  
Major Concerns:  
No.  
  
Minor Concerns:  
-Line 189, should it be 'copolymer stabilizer' or 'copolymer'? It seems like the author defines the copolymer as the stabilizing factor for therapeutics, but reading 'copolymer stabilizer' terminology made some thing it's a stabilizer for copolymer. If possible, I suggest change the description. – We understand the reviewer’s concern but feel that including the additional descriptor helps to indicate what the function of the block copolymer is in the process. Further, since the potentially confusing descriptor is then followed by naming the block copolymer, we feel that it has been sufficiently clarified.   
-Line 234, section 1.4.6. I just feel that it probably needs a lot of practice for one who can depress both solutions at a same rate. I do not know how crucial it is to control the solvent dispensing rate, is there a possible improvement to increase solvent depression consistency and minimize human error? – The reviewer brings up a good point, but we feel that we have addressed this sufficiently in the discussion. Since we are constrained by space, we feel that we are limited to providing references (of which we give many) and the short discussion already included. However, we have added a more explicit statement clarifying that the process is insensitive to flow rate above the transition to turbulent mixing.  
-A general comment, since the procedure uses stabilizer-free THF, I suggest the authors add a THF peroxide test step for a work safety practice.- We added a note where BHT is mentioned regarding peroxide testing.  
-Another general comment, does this method encapsulate all therapeutics into nanoparticle emulations? If not, I suggest adding a purification step or discussion either in the protocol section or in the discussion section. – We agree that noting purification methods and other characterization is important. We have included a number of references describing potential work that could be done with TFF or dialysis to purify solvents or unencapsulated therapeutic away. However, with correct solvent selection, we achieve very high encapsulation rates.  
  
  
**Reviewer #2:**  
Manuscript Summary:  
This manuscript is very detailed and clear to understand with careful reading. The combination of pictures and schematics for the equipment required is very informative for the user in implementing the approach. – We thank the reviewer for their time and care in reviewing.  
  
Major Concerns:  
I have no major concerns.  
  
Minor Concerns:  
Wouldn't using DMSO as the solvent for biologics limit the range of materials that could survive that environment and be encapsulated? - The reviewer brings up an important point. This is an area of focus in our laboratory currently but is far beyond the scope of this protocol as it is a complex subject in and of itself. The protocol is focused on achieving encapsulation of a range of therapeutics and we do not address stabilization techniques purposefully. We can provide patent references if desired.