September 20, 2016

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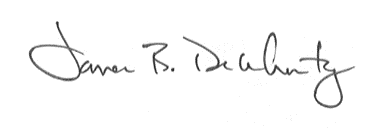
Cambridge, MA 02140

Dear Dr. Singh:

Thank you for your consideration of our submitted manuscript “Targeted Plasma Membrane Delivery of a Hydrophobic Cargo Encapsulated in a Liquid Crystal Nanoparticle Carrier” for publication in *Journal of Visualized Experiments* and for the thorough and thoughtful comments. Attached is a revised version of the manuscript (55181\_R2\_2016\_09\_20.docx) that addresses all of the editorial concerns/reviewer issues raised. Any changes to the manuscript are highlighted as mentioned in the response below. Below is a detailed, by-line item response to each of the critiques raised during the review process. Also attached are revised figures (as .pdf file).

Thank you for your interest in our work and for your consideration for publication of our manuscript in JoVE.

Sincerely,



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Editorial comments:

Response: Any changes made by the authors are highlighted in green in the revised version of the manuscript.

•NOTE: Please download this version of the Microsoft word document (File name: 55181\_R1\_072616) for any subsequent changes. Please keep in mind that some editorial changes have been made prior to peer review.

•Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.

•Formatting: NaN3 is toxic and requires a caution statement.

Response: This has been added to Protocol section 4.7.

•Length exceeds 3 pg; 2.75 pg or less (1 pg minimum) must be highlighted for filming. Steps involving calculations, such as 5.4 & 6.4, should not be highlighted.

Response: ~2.75 of the ~ 4 pgs of protocol text have been highlighted in yellow for filming.

•Please copyedit the manuscript for many grammatical errors. Such editing is required prior to acceptance. Some examples are indicated below:

-5.7 – “The fixed are ready”; “Perform the immediately”; also, please use imperative tense or convert to a note.

-Section 6 note – “is assess”

-Line 471 – “of aspects DiO delivery”

-Line 475 – “was attributed this”

Response: The manuscript has been copy-edited and the appropriate changes have been highlighted in green.

•Additional detail is required:

-2.1.1 – What is the composition of TBE?

-3.3 – What is the composition of complete medium?

Response: The details have been added to Protocol sections 2 and 3.

•Branding: Line 98 – Pluronic®

Response: Pluronic® has been replaced with the chemical name “Poloxamers”.

•Results: Figure 4 – Please indicate the statistical test used.

Response: The data analysis method has been added to section 7.

•Discussion: Please discuss the limitations of the protocol.

Response: Lines 496-500, we have added language that discusses the potential challenge of incorporating other dye and drug cargos that might vary in their degree of hydrophobicity. In these instances, different formulations and ratiometric mixes of the dye/drug cargo will have to be empiraclly tested to determine the optimal mix for successful cargo incorporation into the LCNP.

•If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

Response: The appropriate statement has been added to the figure legend to figures 2-4.

•JoVE reference format requires that the DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

Response: DOIs (for those references that have them) have been added and are highlighted green.

IMPORTANT: Please copy-edit the entire manuscript for any grammatical errors you may find. The text should be in American-English only. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol and the manuscript. Please thoroughly review the language and grammar prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

•NOTE: Please include a line-by-line response letter to the editorial and reviewer comments along with the resubmission.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This is a well written manuscript that is extremely detailed and easy to follow. Having made lipid nanoparticles in the lab before, this is an excellent way to make cross-linked varieties which are more stable.

Major Concerns:

In figure 2 the DIC images are hard to see. Can the contrast be tuned so that the cells appear better in the image?

Response: The DIC image has been tuned so that the cells appear better in the image.

Was a statistical analysis done on figure 4? How relevant is the data?

Response: This information hahs been added in Protocol section 7.

Minor Concerns:

The green highlighted references are not in the same format as the others.

Reponse: These references have been fixed.

Additional Comments to Authors:

N/A

Reviewer #2:

Manuscript Summary:

The authors newly produced a liquid crystal NP (LCNP)-based delivery system for the controlled delivery of a water-insoluble dye, 3′-dioctadecyloxacarbocyanine perchlorate (DiO), from within the NP core to the hydrophobic region of plasma membrane bilayer.

Overall this study has clear-cut purpose and was performed by promising techniques, although methodology is simple to some studies previously reported. I think two minor questions should be answered before publication.

Major Concerns:

N/A

Minor Concerns:

1. How to confirm the DiO concentrations for incubation on cells are same expressed as either the concentration of DiOfree or DiO in the form of DiO-LCNP.

Response: The concentration of the DiO in the DiO-LCNP was determined by using the molar extinction coefficient of a known concentration of DiO in organic solvent. DiOfree and DiOLCNP were adjusted to the same DiO concentrations prior to incubation with the cells.

2. Please show the gel electrophoresis results for confirming successful covalent conjugation of PEG-Chol to the DiO-LCNP surface.

Response: Confirmatory gel electrophoresis images are contained in the original manuscript which is referenced in the protocol section.

Additional Comments to Authors:

N/A