Thank you very much for your kind comments. We have revised the manuscript carefully considering your comments. We hope that following replies meet your comments and we would be glad to respond to any further questions and comments that you may have.

C:\Users\Shinohara\Dropbox\nda\Alkyl_Ru\pictures\151126_reviseexp\Hirahara\Flu_Ru_DOPC\Liposome with crystal.tif

*Comments from Reviewers #3:*

*Comments:*

*Manuscript Summary: The authors have provided protocols for preparation of ruthenium based surfactants followed by their preparation/incorporation into DOPC and DPPC based giant vesicles. It was also demonstrated that fluorescein-DOPC are not suitable for monitoring the morphological changes of vesicles (absence of emission due to metal-ligand charge transfer), whereas rhodamine-DOPC were suitable for this.*

*Major Concerns:*  
*1). The change in morphology suggested by the authors is not significant visually.*

*3). The multi-lamellar architecture claimed by authors cannot be seen visually from the figures. It would help if these changes are clearly labelled in the figures/pictures.*

Answer: As you suggested, morphological changes in Figure 2 is somewhat unclear. There were 30 pictures in the original version in Figure 2. In the revised manuscript, we simplified figure 2 and 6 by omitting the several figures. We have added arrows indicating the morphological changes (budding, size change) in the figures. Also, the font size in the figures was enlarged.

*2). The authors claim that morphology change is seen only in the presence of Na2SO4 and not in NaOH, figures of this claim need to be shown, and brief discussion of the reason for this effect.*Answer: We have added Figure 4 showing the vesicles prepared in an aqueous solution of Na2SO4 or NaOH and their morphological changes under visible light irradiation. Short discussion was added on the differences of the two vesicle dispersions.

*4). The labeling of Figure 2 is unclear. Are figures, 2A, 2B, and 2C from same solution? why do they all have different contrasts?*

Answer: Vesicles in Figure 2A-2C were obtained from the same dispersions. As you suggested, contrasts between 0 min and 16 min are different. This is because we tuned white balance of microscope during the measurements in order to see the interior lamellar structures of vesicles shown in figure 2A.

*Comments from Reviewers #4:*

*Comments:*

*Manuscript Summary: In this paper, the authors have shown that giant vesicles can be fabricated from photo-responsive ruthenium complex. The resultant vesicles can undergo the morphological change after being exposed to visible light due to the photo-isomerization. The experiments were well designed and data are sufficient to support the conclusion. I recommend this work to be published in JVE.  
Additional Comments to Authors:  
Also, It will be interesting to see if this morphological change can be also reversible due to the reversible photo-isomerization which presumably changes the critical packing shape of surfactants back and forth.*

Answer: In a previous article, we reported that the surfactant *proximal*-**2** showed photoisomerization equilibrium between the distal isomer, and the mixture of *proximal* and *distal*-**2** in the photostationary state showed back isomerization to *proximal*-**2** at 45 °C in a mixed aqueous solution in the dark. In the revised version, we carried out photo- and thermal-induced morphological changes of the vesicles. The results of the experiments were added in Figure 5 and brief discussion was added.

Reply to editorial comments

Thank you for fruitful comments on the manuscript. We carefully edited the revised manuscript following the editorial comments. Through the revision of manuscript, the length of the protocol exceeded the limit. Please use the highlighted part for the video article.

***Editorial comments:***

*All of your previous revisions have been incorporated into the most recent version of the manuscript. In addition, Editor may have made minor copy edits to your manuscript and formatting changes to comply with the JoVE format. Please maintain these changes. On the JoVE submission site, you can find the updated manuscript under "file inventory" and download the microsoft word document.* ***Please use this updated version for any future revisions and track all changes using the track changes function in Microsoft Word****.  
  
•Commercial language (brand names of microscopes) was removed from the manuscript. All commercial products should be sufficiently referenced in the Table of Materials.  
  
•Formatting:  
-Please include spaces between all paragraphs and bullet points.  
-Please include spaces between numbers and units.  
-Please revise the legend for figure 1 so that it has a separate title (in bold) followed by a short description of the figure.  
-References – Please abbreviate all journal titles.  
  
•Grammar:  
-Section 1 heading – “Syntheses” should be “Synthesis” for a single chemical.  
-4.3 – Should be “hole punch”  
-4.7 – “Cover with the vesicle dispersion with a cover glass”  
-Line 270 – “Common troubleshooting for the protocols are”  
  
•Additional detail is required:  
-1.4, 2.2.5 – Please provide a citation for chromatography as insufficient detail is provided.  
-1.5 – How is the blue band identified/collected? Is it in fractions eluted from the column? Please provide a literature reference for silica gel chromatography.  
-2.2.5 – How is the purple band identified/collected? Is it in fractions eluted from the column? For TLC, what is the stationary phase? How much sample is spotted on the TLC plate? Is the product visualized in some way?  
-1.6, 2.2.6, 2.3.5 – please provide literature reference(s) for 1H and 13C NMR.   
-3.8 – What is one checking for?  
-4.4 – Wipe with what?  
-4.5 – How is it attached? Is it simply laid on the plate? Are bubbles avoided?  
-5.3 – How far from the plate is the lamp?  
-6.4 – What is the intensity adjusted to?  
  
​•Prior to peer review, the protocol length is at our 3 page limit. If additional details are added to the protocol, please use yellow highlighting to identify a total of 2.75 pages of protocol text (which includes headings and spaces) that should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE's instructions for authors for more clarification and remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.  
  
•Discussion: Please discuss the significance of the technique with respect to alternative methods. Please also discuss the limitations and future applications of the method.*

*•Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammatical errors. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.  
   
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