FINAL SCRIPT: APPROVED FOR FILMING

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Title: Facile Preparation and Photoactivation of Prodrug-Dye Nanoassemblies

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar?**NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES, All Done**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length

Number of Steps: 18
Number of Shots: 37

Introduction

Videographer: Obtain headshots for all authors.

1. **Introductory Interview Statements**

REQUIRED:

1.1. **Zhang Yichi:** This protocol provides a reference on the construction and characterization of photoresponsive drug delivery systems, especially the setup of light irradiation.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.1.2.*

1.2. **Zhang Yichi:** This technique shows the advantages of simple fabrication, high drug-loading capacity, and photo-controllability.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.3.2 and 4.5.1.*

OPTIONAL:

1.3. **Long Kaiqi:** The technique can be used to treat colorectal tumors with the help of optical fibers to deliver light for activating drug release at tumor sites.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 2.3.1.*

1.4. □

Protocol

2. Preparation of IR783/BC Nanoparticles by the Flash Precipitation Method

Videographer: Please capture the shot with labels of all the containers visible during the addition.

2.1. Begin by weighing 10 milligrams of the boron-

dipyrromethene-chlorambucil or BC *(B-C)* prodrug **[1]** and dissolve it in 1 milliliter of Dimethyl sulfoxide or DMSO *(D-M-S-O)* in a 1.5-milliliter microtube **[2]**. Cover the BC solution with foil **[3]**.

2.1.1. Talent weighing 10 mg of the BC prodrug.

2.1.2. Talent adding 10 mg of the BC prodrug BODIPY-Cb to 1 mL of DMSO in a 1.5 mL microtube.

2.1.3. Talent covering the 1.5 mL microtube with foil.

2.2. Then, prepare 300 microliters of 0.4 milligrams per milliliter IR-783 *(I-R-Seven Eight three)* in filtered deionized water in a 1.5-milliliter microtube **[1]**. Place this microtube on a vortex mixer at 1,500 rpm *(R-P-M)* **[2]**. *Videographer: This step is important!*

2.2.1. Talent adding IR-783 in filtered deionized water in a 1.5 mL microtube.

2.2.2. Talent placing the microtube on a vortex mixer.

2.3. Next, with the end of the 20 microliters pipette tip touching the inner wall of the microtube, add 20 microliters of the BC solution to the IR-783 solution over 10 seconds at a constant rate **[1]**. *Videographer: This step is important!*

2.3.1. Talent adding 20 µL of the BC solution to the IR-783 solution using a 20 microliters pipette.

2.4. Place the microtube on the vortex mixer for 30 seconds to obtain the IR783/BC NPs *(I-R-Seven Eight three B-C nanoparticles)* **[1]**. Then, place the nanoparticle solution on a rack fully covered with foil **[2]**. *Videographer: This step is important!*

2.4.1. Talent placing the microtube on the vortex mixer.

2.4.2. Talent placing the nanoparticle solution on a rack fully covered with foil.

2.5. Centrifuge the resulting IR783/BC nanoparticle solution for 10 minutes at 2,000 *g* and 4 degrees Celsius to remove aggregates **[1]**. Collect the supernatant, leaving 20 microliters in the tube to avoid disturbing the pellet, and discard the pellet **[2]**.

2.5.1. Talent placing the IR783/BC NP solution for centrifugation and closing the door.

2.5.2. Talent collecting the supernatant and discarding the pellet.

2.6. After centrifuging the supernatant two times for 30 minutes at 30,000 *g* and 4 degrees Celsius **[1]**, collect the nanoparticle precipitate from both centrifugations **[2]**. Resuspend the nanoparticles in 300 microliters of PBS *(P-B-S)* **[3]**.

2.6.1. Talent placing the supernatant for centrifugation.

2.6.2. Talent collecting the nanoparticle precipitate.

2.6.3. Talent adding 300 µL of PBS to the nanoparticle pellet.

2.7. Quantify the content of IR-783 and BC by high-performance liquid chromatography or HPLC *(H-P-L-C)*, using the elution method **[1-TXT]**. Calculate the prodrug encapsulation efficiency or EE% *(E-E-percent)* and loading capacity or LC% *(L-C-percent)* **[2]**.

2.7.1. Talent quantifying the content of IR-783 and BC by

HPLC. **TXT: Refer to Table 1 for elution method**

Videographer: Please capture talent operating HPLC for quantification.

2.7.2. Talent calculating the prodrug encapsulation efficiency and loading capacity. *Videographer: Please capture the talent looking at the computer screen and calculating EE (%) and LC (%) and the screen showing the calculation.*

Video Editor: Please show below mentioned equations as an inset:

3. Characterization of IR783/BC Nanoparticles

3.1. To measure the average size of the IR783/BC nanoparticles with a dynamic light scattering or DLS *(D-L-S)* instrument, add 200 microliters of IR783/BC nanoparticle solution in a cuvette **[1]** and insert the cuvette in the holder for measurement **[2]**.

3.1.1. Talent adding 200 µL of IR783/BC NP solution in a cuvette.

3.1.2. Talent inserting the cuvette in the holder of the DLS instrument for measurement.

3.2. Set the measurement type as 'size' and the measurement temperature as 25 degrees Celsius. Perform three measurements with a duration of 20 seconds for each measurement **[1]**.

3.2.1. SCREEN: 64677_Screenshot_1: 00:09 to 00:16, then

00:22 to 00:41 then, 00:48 to 00:49 then, 00:54 to 00:55, then 01:06 to 01:07, Then 1:35-1:36. *Video Editor: please speed up the video as required.*

Videographer: Please capture a few extra shots of talent operating the instrument and talent looking at the screen and setting the parameters as a backup.

3.3. To measure the surface charge of the IR783/BC nanoparticles with the DLS instrument, dilute 25 microliters of IR783/BC nanoparticle solution with 725 microliters of deionized water in a 1.5-milliliter microtube **[1]**. Add the solution into a zeta-potential test cuvette **[2]**. Place the cuvette in the sample groove. Cap the sample groove **[3]**.

3.3.1. Talent adding 25 µL of NP solution in a 725 µL of deionized water in a 1.5-mL microtube

3.3.2. Talent adding the solution into a zeta-potential test cuvette.

3.3.3. Talent placing the cuvette in the sample groove and covering the sample groove.

3.4. Next, set the measurement type as 'zeta-potential' and the temperature as 25 degrees Celsius. Perform 10 measurements **[1]**.

3.4.1. SCREEN: 64677_Screenshot_2: 00:01 to 00:05, then 00:14 to 00:15, then 00:18 to 00:22, then 00:26 to 00:31, then 00:34 to 00:35, then 00:40 to 00:41, then 01:11 to 01:14. *Video Editor: please speed up the video as required.*

3.5. Once done, prepare the samples for transmission electron

microscopy or TEM *(T-E-M)* imaging by adding 10 microliters of IR783/BC nanoparticle solution on a piece of the holey carbon film on a copper grid of 300 mesh **[1]** and removing 7 microliters from the holey carbon film **[2]**.

3.5.1. Talent adding 10 µL of IR783/BC nanoparticle solution on a piece of the holey carbon film on a copper grid of 300 mesh.

3.5.2. Talent removing 7 µL of nanoparticle solution from a piece of the holey carbon film.

3.6. Leave 3 microliters of solution on the film overnight for auto-evaporation **[1]**.

3.6.1. The film with 3 µL solution placed for evaporation on a platform being seen.

4. Photoactivation of IR783/BC Nanoparticles

4.1. Set up an LED *(L-E-D)* lamp at 530 nanometers with an iron stand so that the light directly faces the operating floor **[1]**. Place an integrating sphere photodiode photometer directly under the LED lamp **[2]**.

4.1.1. Talent setting up an LED lamp with an iron stand. OR The LED lamp with an iron stand being seen.

4.1.2. Talent placing an integrating sphere photodiode photometer under the LED lamp.

4.2. Turn on the LED lamp **[1]** and open the cap of the photometer **[2]**. Record the irradiance, set the lamp parameters using the associated software, and adjust the input current in the milliampere to set the irradiance as 50 milliwatts per square centimeter **[3]**. *Videographer: This*

step is important!

4.2.1. Talent turning on the LED lamp. OR The LED lamp being turned on.

4.2.2. Talent opening the cap of the photometer.

4.2.3. SCREEN: 64677_Screenshot_3: 00:09 to 00:13, Then 00:22 to 00:23, then 00:27 to 00:30, then 00:35 to 00:41, then 00:58 to 01:02. *Video Editor: please speed up the video as required.*

4.3. Dilute the IR783/BC nanoparticles solution with deionized water to 50 micromolar based on BC concentration **[1]**. Add 200 micromolar of the IR783/BC nanoparticles solution into a 1.5-milliliter microtube **[2]**. Place the tube on a foam block having a groove fitting the size of the microtube and at the same height as the photometer **[3]**. *Videographer: This step is important!*

4.3.1. Talent diluting IR783/BC NPs solution with deionized water.

4.3.2. Talent adding 200 µL of the IR783/BC NP solution into a 1.5-mL microtube.

4.3.3. Talent placing the tube on a foam block.

4.4. Open the cap of the tube **[1]**. Switch on the LED lamp and irradiate the nanoparticle solution for 1, 2, 3, 5, 7, and 10 minutes **[2]**. *Videographer: This step is important!*

4.4.1. Talent opening the cap of the tube

4.4.2. The LED being switched **ON,** and the nanoparticles being irradiated.

4.5. After light irradiation, quantify BC *(B-C)* consumption and

Cb *(C-B)* release by HPLC *(H-P-L-C)* **[1]** and calculate the remaining BC and Cb release **[2]**.

4.5.1. Talent quantifying BC consumption and Cb release on HPLC.

4.5.2. TEXT ON PLAIN BACKGROUND:

Video Editor: Please show the equation on the screen

Results

5. **Results: Fabrication and Characterization of a Photoresponsive Prodrug-Dye Nano Assembly**

5.1. IR783/BC nanoparticles were successfully fabricated in this study using a flash precipitation method **[1]**. The synthesized nanoparticles were presented as a purple solution **[2]**, while the aqueous solution of IR783 was blue **[3]**.

5.1.1. LAB MEDIA: Figure 4A.

5.1.2. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the last purple vial representing IR783/BC nanoparticles.*

5.1.3. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the blue vial representing free IR783.*

5.2. The IR783/BC nanoparticles exhibited an average size of 87.22 nanometers with a polydispersity index or PDI *(P-D-I)* of 0.089, demonstrating a narrow size distribution **[1]**.

5.2.1. LAB MEDIA: Figure 4B.

5.3. The surface charge was approximately minus 29.8 millivolts, indicating the negatively charged sulfonate groups of IR783 **[1]**. The nanoparticle's size was maintained at 85 nanometers for at least 48 hours after fabrication, while its PDI remained less than 0.2. **[2]**.

5.3.1. LAB MEDIA: Figure 4C.

5.3.2. LAB MEDIA: Figure 4D.

5.4. No significant change was observed in the size distribution at 0, 24, and 48 hours after fabrication **[1]**.

5.4.1. LAB MEDIA: Figure 4E.

5.5. Aggregates and fragments **[1]** were observed after light irradiation **[2]**. Size and distribution changes were observed after 3 and 5 minutes of light irradiation **[3]**.

5.5.1. LAB MEDIA: Figures 5A, B.

5.5.2. LAB MEDIA: Figures 5A, B. *Video Editor: Please emphasize 5B.*

5.5.3. LAB MEDIA: Figure 5C.

5.6. Prodrug **[1]** BC was photocleaved in 10 minutes **[2]**. Meanwhile, chlorambucil was released with a recovery efficiency of around 22% within the same period **[3]**.

5.6.1. LAB MEDIA: Figure 5D, E.

5.6.2. LAB MEDIA: Figure 5D, E. *Video Editor: Please emphasize BC peaks in 5D and the red line in figure 5E.*

5.6.3. LAB MEDIA: Figure 5D, E. *Video Editor: Please emphasize Cb peaks in 5D and the green line with*

the triangles representing released Cb in figure 5E

5.7. The IR783/BC nanoparticles displayed significant cytotoxicity **[1]** on human colorectal tumor cells HCT116 *(H-C-T-1-1-6)* under light irradiation at 530 nanometers **[2]** compared with the non-irradiation group **[3]**.

5.7.1. LAB MEDIA: Figure 6.

5.7.2. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the light purple line representing IR783/BC NPs + hv*

5.7.3. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the red line representing IR783/BC NPs* □

Conclusion

6. Conclusion Interview Statements

6.1. **Zhang Yichi:** It is important to touch the inner wall of the microtube with the end of the pipette tightly and place the microtube on the vortex stably.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1. and 2.4.1.*