

Submission ID #: 70445

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21485773>

Title: Synthesis, Hemoglobin Encapsulation and Biorthogonal PEGylation in Hierarchically Porous UiO-66 Nanoparticles for Oxygen Delivery Applications

Authors and Affiliations:

Weiguang Jin and Leticia Hosta-Rigau

Department of Health Technology, Technical University of Denmark

Corresponding Authors:

Leticia Hosta-Rigau (leri@dtu.dk)

Email Addresses for All Authors:

Weiguang Jin (weig@dtu.dk)

Leticia Hosta-Rigau (leri@dtu.dk)

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? **NO**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

In same building but different lab needs 5 min walking

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 15

Number of Shots: 42

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Weiguang Jin:** The focus of our research is to develop a stable MOF-based nanoparticle for hemoglobin encapsulation and oxygen delivery.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Weiguang Jin:** Hierarchically porous MOFs overcome pore-size limitations for hemoglobin loading, while PEGylation enhances nanoparticle stability in physiological environments.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Weiguang Jin:** This protocol enables the synthesis of hierarchically porous MOF nanoparticles, hemoglobin loading, surface PEGylation, and evaluation of oxygen-release functionality.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer's NOTE: There were 2 takes in 1 shot for 1.3.1.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Weiguang Jin:** Using this protocol, additional physicochemical characterization and functional studies can be conducted, including in vitro analyses of oxygen release and nanoparticle biocompatibility.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Weiguang Jin:** Future studies can focus on preserving hemoglobin functionality for longer durations, such as incorporating antioxidant components to prevent oxidative degradation.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

NOTE: All on-screen texts that originally served as supplemental instructions has been moved to voiceover in accordance with the updated guidelines.

2. Azide Functionalization of HP-UiO-66-NH₂ NPs (HP-UiO-66-N₃ NPs)

Demonstrator: Weiguang Jin

- 2.1. To begin, accurately weigh 50 milligrams of prepared hierarchically porous-UiO-66 (*U-I-O-Sixty-Six*) amine nanoparticles into a 25-milliliter round-bottom flask [1]. Add 10 milliliters of tetrahydrofuran to the flask [2]. Then, ultrasonicate the mixture for 5 minutes to fully disperse the particles [3], producing a uniform opaque suspension [4].
 - 2.1.1. WIDE: Talent weighing HP-UiO-66-NH₂ nanoparticles and transferring them into a 25-milliliter round-bottom flask. *Videographer: Please film a CU shot as well*
 - 2.1.2. Talent adding tetrahydrofuran to the round-bottom flask.
 - 2.1.3. Talent placing the round-bottom flask in an ultrasonic bath for sonication.
Videographer's NOTE: There were 2 takes for shot 2.1.3. Use the 2nd one.
 - 2.1.4. Close shot of the round-bottom flask showing an opaque but uniform suspension.
- 2.2. Place the round-bottom flask in an ice-water bath on a magnetic stirrer set to 400 rpm [1]. Using a glass syringe, add 0.4 milliliters of tert-butyl nitrite dropwise over 5 minutes while maintaining the temperature below 5 degrees Celsius [2].
Videographer's NOTE: 2.2.1 to 2.3.3 was covered in 1 shot.
 - 2.2.1. Talent placing the round-bottom flask into an ice-water bath positioned on a magnetic stirrer.
 - 2.2.2. Talent adding tert-butyl nitrite dropwise into the flask with a glass syringe.
- 2.3. Immediately add 0.35 milliliters of trimethylsilyl azide dropwise under the same cold conditions [1]. A mild effervescence may appear, indicating nitrosation progress [2]. Then, wrap the ice-water bath in aluminum foil to protect the reaction from light [3].
 - 2.3.1. Talent adding trimethylsilyl azide dropwise into the reaction flask with a syringe while the flask remains in the ice-water bath.

2.3.2. Close shot of mild effervescence appearing in the reaction mixture.

2.3.3. Talent wrapping aluminum foil around the ice-water bath.

2.4. Continue stirring the reaction mixture in the ice-water bath overnight, then switch off the lights and close the front glass. [1]. After washing and drying the product, use FTIR (F-T-I-R) spectroscopy to verify the chemical transformation [2].

2.4.1. Talent placing the reaction flask in the ice-water bath on the magnetic stirrer, switching off the light and closing the front glass.

Videographer's NOTE: Talent said 2.4.1 was included in the previous shot. So, the switching off the light and closing the front glass was filmed as 2.4.1.

2.4.2. Talent places the sample in the spectroscope.

3. Hemoglobin Loading and Quantification

3.1. Transfer 5 milligrams of hierarchically porous UiO-66-azide nanoparticles into a 2-milliliter centrifuge tube [1]. Add 0.5 milliliters of HEPES (Hepes) buffer [2] and sonicate for 2-5 minutes [3]. Add 0.5 milliliters of HEPES buffer containing 20 milligrams per milliliter hemoglobin and gently invert the tube several times to fully disperse the nanoparticles [4].

3.1.1. Talent transferring HP-UiO-66-N3 nanoparticles into a 2 milliliter centrifuge tube.

Videographer's NOTE: Use the second take of 3.1.12 after 0:34 in order to not see the screen in the bottom.

3.1.2. Talent adding HEPES buffer to the centrifuge tube.

3.1.3. Talent placing the tube in the sonicator.

3.1.4. Talent adding HEPES buffer containing hemoglobin into tube and gently inverting the centrifuge tube several times to disperse the nanoparticles.

3.2. Now, place the tube on a thermoshaker and incubate at room temperature with shaking at 1,000 rpm for 2 hours [1].

3.2.1. Talent placing the centrifuge tube onto the thermoshaker.

3.3. After incubation, centrifuge the tube at 13,500 *g* for 5 minutes at room temperature to pellet the resulting hemoglobin at hierarchically porous UiO 66 azide nanoparticles [1]. Then, carefully collect the supernatant for quantification of untrapped hemoglobin [2].

3.3.1. Talent placing the centrifuge tube into the centrifuge.

- 3.3.2. Talent transferring the supernatant from the centrifuge tube into a new tube.
- 3.4. Wash the pellet gently thrice, each time with 1 milliliter of ultrapure water by pipetting up and down to remove loosely adsorbed hemoglobin [1]. Then, carefully collect the supernatant of each wash for quantification of untrapped hemoglobin [2]. Resuspend the final pellet in 0.5 milliliters of ultrapure water and store in a refrigerator prior to characterization [3].
- 3.4.1. Talent adding ultrapure water to the centrifuge tube containing the nanoparticle pellet. ~~TXT: Repeat wash 2x~~
Videographer's NOTE: Shot 3.4.1 was filmed along with shot 3.3.2.
- 3.4.2. Talent collects the supernatant and transfers it into a fresh tube.
- 3.4.3. Talent resuspending the final nanoparticle pellet in ultrapure water. ~~TXT: Keep samples in refrigerator to prevent oxidation.~~
- 3.5. Use the sodium lauryl sulfate hemoglobin assay to determine untrapped hemoglobin in the collected supernatants [1]. Pipette 10 microliters of each supernatant into a 96-well microplate [2]. Add 100 microliters of sodium lauryl sulfate reagent [3] and incubate for 5 minutes at room temperature in the dark [4]. Using an ultraviolet-visible spectrophotometer, measure absorbance at 412 nanometres [5].
- 3.5.1. Talent at the bench with the sodium lauryl sulfate hemoglobin assay reagent placed in front. **Videographer's NOTE: 3.5.1, 3.5.2 and 3.5.3 are combined in 1 shot**
- 3.5.2. Talent pipetting supernatant into wells of a 96-well microplate.
- 3.5.3. Talent adding sodium lauryl sulfate reagent to the wells.
- 3.5.4. Shot of the microplate incubating in the dark at room temperature.
- 3.5.5. Talent placing the microplate into a ultraviolet-visible spectrophotometer to read absorbance.
- 3.6. Afterwards, prepare serial dilutions of hemoglobin stock in HEPES buffer to generate a standard curve [1]. Transfer the prepared dilutions into the prepared 96-well plate to generate a standard curve for converting absorbance readings into hemoglobin concentrations [2].
- 3.6.1. Serial dilution of stock of hemoglobin (20 mg mL^{-1}) in 2 mL centrifuge tubes
Videographer's NOTE: 3.6.1, 3.6.2 are combined in 1 shot.
- 3.6.2. Talent transferring hemoglobin at different concentration into the same 96-well microplate.

4. Fabrication of Hb@HP-UiO-66-PEG NPs: PEGylation via SPAAC

4.1. Transfer 10 milligrams of hemoglobin at hierarchically porous-UiO-66-azide nanoparticles into a 2-milliliter centrifuge tube containing 0.5 milliliters of ultrapure water [1] and sonicate for 2-5 minutes [2]. Add 20 milligrams of Dibenzylcyclooctyne modified with polyethylene glycol to achieve an equimolar ratio of polyethylene glycol to nanoparticles [3]. Gently vortex the tube until the mixture is homogenized [4].

4.1.1. Talent transferring Hb@HP-UiO-66-N3 nanoparticles into a 2 milliliter centrifuge tube containing ultrapure water.

4.1.2. Talent sonicating the tube for 2-5 minutes.

Videographer's NOTE: 4.1.3, 4.1.4 and 4.2.1 are combined in 1 shot.

4.1.3. Talent adding the DBCO-PEG solution to the centrifuge tube.

4.1.4. Talent vortexing the centrifuge tube to mix the suspension.

4.2. Place the tube on a thermoshaker and incubate at room temperature with shaking at 1,000 rpm for 1 hour [1]. Then transfer the tube into a refrigerator maintained at 4 degrees Celsius [2].

4.2.1. Talent placing the centrifuge tube onto the thermoshaker.

4.2.2. Talent placing the centrifuge tube into the refrigerator.

4.3. Centrifuge the tube at 12,000 *g* for 5 min at room temperature to pellet the polyethylene glycol-modified hemoglobin at hierarchically porous-UiO-66-Polyethylene glycol nanoparticles [1]. Then, discard the supernatant containing unreacted polyethylene glycol [2].

4.3.1. Talent placing the centrifuge tube into the centrifuge.

Videographer's NOTE: 4.3.2, 4.4.1 and 4.4.2 are combined in 1 shot.

4.3.2. Talent discarding the supernatant from the centrifuge tube.

4.4. Wash the pellet three times with 2 milliliter ultrapure water each by pipetting up and down [1] and centrifuge under the same conditions as demonstrated earlier [2].

4.4.1. Talent adding ultrapure water to the centrifuge tube containing the nanoparticle pellet and pipetting up and down.

4.4.2. Talent placing the centrifuge tube into the centrifuge. ~~TXT: Repeat wash 3x~~

4.5. Resuspend the purified polyethylene glycol-modified hemoglobin at hierarchically porous UiO-66-Polyethylene glycol nanoparticles in 2 milliliters of ultrapure water [1]. Store the suspension at 4 degrees Celsius [2]. The resulting colloid appears homogeneous and slightly opalescent, indicating good dispersion stability [3]. Then, confirm successful PEGylation (*Pegylation*) by dynamic light scattering or DLS (*D-L-S*) [4].

4.5.1. Talent resuspending the purified nanoparticle pellet in ultrapure water.

4.5.2. Talent placing the centrifuge tube in a refrigerator.

4.5.3. Close shot of the centrifuge tube showing a homogeneous and slightly opalescent nanoparticle suspension.

4.5.4. Talent loading the sample in the spectroscopy DLS equipment.

Results

5. Results

5.1. Surface amino groups were successfully converted to azides [1], as demonstrated by the appearance of a characteristic asymmetric azide stretching band at 2122 (*Two-One-Two-Two*) centimeters inverse in the FTIR spectrum of hierarchically porous UiO-66-azide nanoparticles [2], which was absent in hierarchically porous UiO-66-amine nanoparticles [3].

5.1.1. LAB MEDIA: Figure 4A.

5.1.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the peak labelled 2122 on the spectrum for “HP-UiO-66-N₃ NPs”.*

5.1.3. LAB MEDIA: Figure 4A. *Video editor: Show the spectrum labelled “HP-UiO-66-NH₂ NPs” and indicate that the peak at 2122 is absent.*

5.2. Hemoglobin encapsulation was visually indicated by a distinct yellow-to-red color change [1].

5.2.1. LAB MEDIA: Figure 5A. *Video editor: Show the particle labeled “Hb@HP-UiO-66-N₃ NPs” with the red color.*

5.3. Nitrogen uptake decreased after hemoglobin loading, indicating occupation of mesopores by hemoglobin molecules [1]. The zeta potential decreased from 42.9 millivolts for hierarchically porous UiO-66-azide nanoparticles [2] to 20.4 millivolts for hemoglobin at hierarchically porous UiO-66-azide nanoparticles after hemoglobin encapsulation [3].

5.3.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the lower curve labelled “Hb@HP-UiO-66-N₃ NPs” compared with the higher curve labelled “HP-UiO-66-N₃ NPs”.*

5.3.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the bar labeled 42.9 millivolt above the green particle icon.*

5.3.3. LAB MEDIA: Figure 5C. *Video editor: Highlight the bar labeled 20.4 millivolt above the orange particle icon.*

5.4. Successful polyethylene glycol conjugation was confirmed by the disappearance of the azide stretching vibration at 2122 centimetres inverse [1], and the appearance of polyethylene glycol-associated signals in the FTIR spectrum [2]. DLS analysis showed an increase in hydrodynamic diameter from approximately 114.9 to 140.8 nanometers

after PEGylation, while maintaining a low PDI (*P-D-I*) [3].

- 5.4.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the peak labeled 2122 in the spectrum for "Hb@HP-UiO-66-N₃ NPs".*
- 5.4.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the additional peaks in the spectrum labeled "Hb@HP-UiO-66-PEG NPs".*
- 5.4.3. LAB MEDIA: Figure 6C. *Video editor: Highlight the DLS size distribution labeled "Hb@HP-UiO-66-PEG NPs".*

Pronunciation Guides:

1. Azide
Pronunciation link: <https://www.merriam-webster.com/dictionary/azide>
IPA: /'eɪ.zaid/
Phonetic Spelling: ay·zide
2. Functionalization
Pronunciation link: <https://www.merriam-webster.com/dictionary/functionalization>
IPA: /ˌfʌŋkʃənələ'zeɪʃən/
Phonetic Spelling: fuhngk·shuh·nuh·luh·zay·shuhn
3. Hierarchically
Pronunciation link: <https://www.merriam-webster.com/dictionary/hierarchically>
IPA: /ˌhaɪə'rɑːrkɪkli/
Phonetic Spelling: hy·uh·raar·ki·klee
4. Tetrahydrofuran
Pronunciation link: <https://www.merriam-webster.com/dictionary/tetrahydrofuran>
IPA: /ˌtetrəˌhaɪdroʊ'fjʊərən/
Phonetic Spelling: te·truh·hy·droh·fyoo·ran
5. Ultrasonicate
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasonicate>
IPA: /ˌʌltrə'sɑːnɪˌkeɪt/
Phonetic Spelling: uhl·truh·saa·nuh·kayt
6. Opaque
Pronunciation link: <https://www.merriam-webster.com/dictionary/opaque>
IPA: /oʊ'peɪk/
Phonetic Spelling: oh·payk
7. Suspension
Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>
IPA: /sə'spɛnʃən/
Phonetic Spelling: suh·spen·shuhn
8. Tert-butyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/tert-butyl>
IPA: /'tɜːrt 'bjuːtəl/
Phonetic Spelling: turt·byoo·tuhl
9. Trimethylsilyl
Pronunciation link: No confirmed link found
IPA: /ˌtraɪˌmɛθɪl'sɪlaɪl/
Phonetic Spelling: try·meth·uhl·sy·lile
10. Effervescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/effervescence>
IPA: /ˌɛfər'vesəns/
Phonetic Spelling: ef·ur·ves·uhns

11. Nitrosation
Pronunciation link: No confirmed link found
IPA: /ˌnaɪtroʊˈseɪʃən/
Phonetic Spelling: ny-troh-say-shuhn
12. Spectroscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectroscopy>
IPA: /ˈspekˈtrɑːskəpi/
Phonetic Spelling: spek-traa-skuh-pee
13. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsentrəˌfjuːdʒ/
Phonetic Spelling: sen-truh-fyooj
14. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuːpərˈnextənt/
Phonetic Spelling: soo-pur-nay-tuhnt
15. Quantification
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantification>
IPA: /ˌkwɑːntəfəˈkeɪʃən/
Phonetic Spelling: kwon-tuh-fuh-kay-shuhn
16. Ultrapure
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrapure>
IPA: /ˌʌltrəˈpjʊr/
Phonetic Spelling: uhl-truh-pyoor
17. Hemoglobin
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemoglobin>
IPA: /ˌhiːməˈɡloʊbɪn/
Phonetic Spelling: hee-muh-gloh-bin
18. Spectrophotometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
IPA: /ˌspektrəfoʊˈtɑːmətər/
Phonetic Spelling: spek-troh-foh-taa-muh-tur
19. Polyethylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyethylene>
IPA: /ˌpɑːliˈeθəliːn/
Phonetic Spelling: pah-lee-eth-uh-leen
20. Dibenzylcyclooctyne
Pronunciation link: No confirmed link found
IPA: /daɪˌbenziːlˌsaɪkloʊˈɑːktɑɪn/
Phonetic Spelling: dye-ben-zeel-sy-kloh-ok-tyne
21. PEGylation
Pronunciation link: <https://www.merriam-webster.com/dictionary/PEGylation>

IPA: /ˌpɛɡɪˈleɪʃən/

Phonetic Spelling: peg·ih·lay·shuhn

22. Homogenized

Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenized>

IPA: /həˈmɑːdʒəˌnaɪzd/

Phonetic Spelling: huh·maa·juh·nyzd

23. Colloid

Pronunciation link: <https://www.merriam-webster.com/dictionary/colloid>

IPA: /ˈkɔːlɔɪd/

Phonetic Spelling: kol·oyd

24. Opalescent

Pronunciation link: <https://www.merriam-webster.com/dictionary/opalescent>

IPA: /ˌɒpəˈlesənt/

Phonetic Spelling: oh·puh·les·uhnt

25. Hydrodynamic

Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrodynamic>

IPA: /ˌhaɪdrəʊdaɪˈnæmɪk/

Phonetic Spelling: hy·droh·dy·nam·ik

26. Mesopores

Pronunciation link: No confirmed link found

IPA: /ˈmezoʊpɔːrz/

Phonetic Spelling: meh·zoh·porz

27. Zeta

Pronunciation link: <https://www.merriam-webster.com/dictionary/zeta>

IPA: /ˈzeɪtə/

Phonetic Spelling: zay·tuh

28. Conjugation

Pronunciation link: <https://www.merriam-webster.com/dictionary/conjugation>

IPA: /ˌkɔːndʒəˈgeɪʃən/

Phonetic Spelling: kon·juh·gay·shuhn