Chapter 8

Synthesis of Metal–Organic Frameworks on Tobacco Mosaic Virus Templates

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Abstract

Tobacco mosaic virus (TMV) has long been exploited as a robust biological scaffold for organic/inorganic modification owing to its anisotropic structure and chemically addressable amino acid residues on both the exterior and interior. We present the fabrication of a crystalline microporous metal–organic framework (MOF) shell on the exterior of TMV, which retains its rod-like morphology, and produces uniformly formed core–shell structures with high accessible surface area and pore volume. We also describe an exfoliation method that can recover the intact viral particle from the core–shell composite.

Key words Metal–organic frameworks, MOFs, Virus-like particles, VLPs, SURMOFs, Self-assembly, Biomimetic mineralization, Templated crystal growth, Tobacco mosaic virus, Virus nanotechnology, Chemical virology

1 Introduction

The preparation of synthetic anisotropic polymeric structures with near exact size control in the hundreds of nanometer range is a challenge hitherto unrealized. This is unfortunate, as soft anisotropic templates would be of considerable use in optics, nanoscale electronics, and biomedicine. While controlled assembly via supramolecular templating [1] has been able to produce rigid anisotropic rods many nanometers long and with zero polydispersity, the practicality of expanding these synthetic methods to the hundreds of nanometer regimes seems unlikely. Access to these seemingly privileged structures, however, is of little effort to nature. Tobacco mosaic virus (TMV, illustrated in Fig. 1a) is a rod-shaped virus composed of 2130 coat proteins that self-assemble around a single strand of RNA [2]. The self-assembled product is a tubular structure of approximately 300 × 18 nm with an inner longitudinal channel 4 nm in diameter. The coat protein is chemically addressable by diazonium coupling to surface exposed tyrosine residues on the exterior whilst the inner surface can be

Andrew K. Udit (ed.), *Protein Scaffolds: Design, Synthesis, and Applications*, Methods in Molecular Biology, vol. 1798, https://doi.org/10.1007/978-1-4939-7893-9_8,

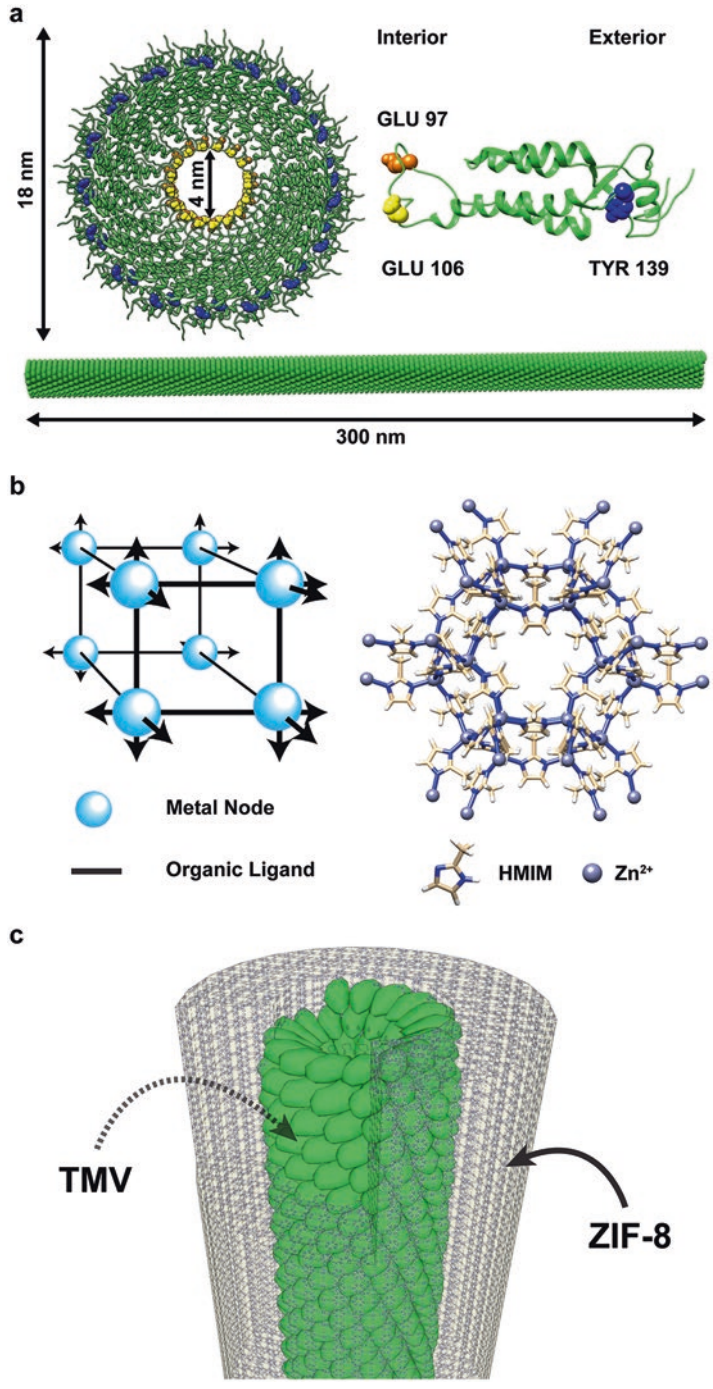

Fig. 1 (**a**) Simulated proteinaceous models that illustrate the morphology and structure of TMV: top-down view (left), single coat protein monomer (right), and integrated rod-like viral particle (bottom). (**b**) Illustration of MOFs. Left: a simplified representation of a typical MOF unit cell that repeats infinitely in all directions to form a porous matrix. Right: Crystal structure of the unit cell of zeolitic imidazolate framework-8 (ZIF-8), which is crystallized on the surface of TMV. (**c**) Schematic illustration of TMV@ZIF-8. Figure 1c is reproduced from Ref. [20] with permission from Wiley

modified using traditional amide coupling reactions to surface exposed glutamic acid residues [3]. This high aspect ratio tubular protein nanoparticle possesses extraordinary stability in a broad range of temperatures (up to 60 °C) and pH (2–10) [4]. Because these structures are templated by their RNA, it has been possible to precisely change the length of the viral rods via straightforward methods [5].

In the early 2000s, several pioneering studies demonstrated TMV's role as a versatile biological scaffold for directed synthesis of inorganic nanoparticles or hollow nanorods [4, 6–10]. Shenton et al. [4], for instance, investigated the capability of TMV to act as a template under broad mineralization conditions with various metal sources. They demonstrated that a uniform thin layer of SiO_2, PbS, CdS, and iron oxide nanoparticles could be coated on the outer surface of TMV at pH values ranging from 2.5 to 9. In another example, Dujardin et al. [7] used solutions of different pH to induce the formation of sub-10 nm metal nanoparticles selectively on either the exterior or interior surface of TMV by exploiting the differences in the pKa's of surface-exposed amino acids on either the outer or the inner surfaces. Inspired by these preliminary results, researchers have prepared metal/metal oxide-coated TMV nanorods and applied them in device fabrication and imaging applications [6, 9, 10].

Concurrent to and independent of the research focusing on metal/metal oxide deposition on viral nanoparticles, a new type of highly crystalline porous metal coordination polymer was being developed called "metal–organic frameworks" (Fig. 1b). Metal–organic frameworks (MOFs) possess three-dimensionally ordered channels composed of either micropores or mesopores and typically possess a high surface area [11]. Very recently, researchers have shown that it is possible to prepare MOFs under biocompatible conditions and have demonstrated that these advanced synthetic strategies allow MOF-coated biomaterials, such as enzymes, to retain their selectivity, catalytic efficiency, and stability even when the enzyme is buried within a single crystal of MOF [12–20]. Further, the MOF crystal affords incredible protection to the enzyme against proteolysis and denaturation as a result of exposure to harsh solvents or high temperatures [12].

From our initial perspective, therefore, the benefits for fabrication of MOFs on TMV were apparent: the porous materials have been widely employed in mass storage, mass transfer and catalysis applications [21–24]; the porous structure could protect the inlaid TMV from environmental attacks as well as leave space and pathways for surface exposed tyrosine residues for chemical modification under harsh synthetic organic conditions.

Here, we present the synthesis of MOF-encapsulated TMV bionanoparticles, which possesses a crystalline yet porous MOF

shell that closely packs on the exterior of TMV [20] and is schematically shown in Fig. 1c. We demonstrated that zeolitic imidazolate framework-8 (ZIF-8) [25], a typical MOF with excellent thermal and chemical stability, can be homogeneously coated on the TMV and the thickness of the ZIF-8 layer can be tuned by changing the synthetic conditions. The as-obtained TMV@ZIF-8 bionanoparticles can be characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), powder X-ray diffraction (PXRD), and gas sorption analysis. A key benefit of using a metal-based coordination polymer like a ZIF is that the coating is easily destroyed using a strong chelator like ethylenediamine tetraacetic acid (EDTA). This method returns the ZIF crystal to its small monomeric components neatly and rapidly. This allows us to reversibly capture and release the TMV from its protective shell. Herein we also describe the protocol for recovering the intact viral particles following exfoliation and dissolution of the ZIF coating with an aqueous solution of EDTA.

2 Materials

All chemicals are analytical grade and used without further purification. All aqueous solutions were prepared with ultrapure water (18.2 MΩ). All buffer solutions and TMV solutions are stored at 4 °C.

1. Tobacco seeds (*Nicotiana benthamiana*) from healthy plants.
2. Dedicated plant room at 25 °C, 65% humidity, and lighting period of 16 h.
3. 0.1 M Phosphate buffer: Dissolve 17.418 g of K_2HPO_4 in 100 mL ultrapure water to obtain a 1.0 M solution. Dissolve 13.609 g of KH_2PO_4 in 100 mL ultrapure water to obtain a 1.0 M solution. Mix 30.75 mL of the 1.0 M K_2HPO_4, 19.25 mL of the 1.0 M KH_2PO_4 mL and 450 mL ultrapure water to obtain a 0.1 M phosphate buffer pH 7.4. The pH of the buffer solution should be confirmed by pH meter. 0.01 M phosphate buffer can be prepared by diluting the 0.1 M solution.
4. Silicon carbide powder.
5. Extraction solution: Add 1 mL of β-mercaptoethanol to 499 mL of 0.1 M phosphate buffer to make a final volume of 500 mL. The concentration of β-mercaptoethanol is 0.2% (v/v).
6. Hamilton Beach Type B31 blender.
7. Cheesecloth.
8. Chloroform.

9. n-butanol.
10. Sodium chloride.
11. PEG-8000.
12. Triton X-100.
13. 40% (w/v) aqueous sucrose solution.
14. Thermo Scientific Sorvall Lynx 4000 Centrifuge or equivalent.
15. Thermo Scientific Sorvall wX+ Ultra Series Centrifuge or equivalent.
16. Fiberlite™ F10-4 × 1000 LEX Rotor.
17. Fiberlite™ F37 L-8 × 100 Fixed-Angle Rotor.
18. Beckman Coulter Microfuge 16 Centrifuge with FX241.5P Rotor.
19. G3000SW$_{XL}$ column on an Agilent 1100 series HPLC system.
20. Thermo Scientific NanoDrop 2000 Spectrophotometer.
21. GE Health Care PD midi Trap G-25 column.
22. ZIF-8 metal precursor solution: 20 mM zinc acetate in ultrapure water (*see* **Note 1**).
23. ZIF-8 ligand precursor solution: 400 mM 2-methylimidazole prepared in ultrapure water (*see* **Note 1**).
24. EDTA exfoliation solution (0.1 M, pH 7.0): Add 2.9224 g EDTA to 90 mL 0.1 M KOH aqueous solution, use a stir plate and magnetic stir bar to achieve best dissolution. Measure the pH of the as-prepared solution by pH meter and add 2.0 M HCl to adjust the pH to 7.0 if needed. Finally adjust the total volume to 100 mL with ultrapure water.
25. Negative staining solution: 2% (wt%) uranyl acetate in ultrapure water. The as-prepared solution should be stored at 4 °C (*see* **Note 2**).
26. Silicon wafer for scanning electron microscopy.
27. Acetone.
28. Methanol.
29. Double-sided copper tape for scanning electron microscopy.
30. Zeiss Supra 40 scanning electron microscope.
31. FCF200-Cu sample grid for transmission electron microscopy.
32. FEI Tecnai G2 Spirit Biotwin transmission electron microscope.
33. Bruker D8 Advance powder X-ray diffractometer.
34. Micromeritics ASAP 2020 surface area analyzer.

3 Methods

3.1 TMV Production and Purification

1. TMV is propagated in *N. benthamiana* in a dedicated plant room at 25 °C, 65% humidity and lighting period of 16 h. The tobacco plants are watered every other day (*see* **Note 3**).
2. When the size of the leaf is about 5 cm wide, conduct the infection on the day that the plants are supposed to be watered. Tap a small amount of silicon carbide with finger and sprinkle the powder onto the leaves. Add 30 μL of 0.17–0.27 mg/mL TMV in phosphate buffer to each leaf. Gently rub the leaf to spread the virus solution and let it enter the leaf (*see* **Note 4**).
3. Harvest the leaves after 7–10 days of infection. Weigh the leaves and store them in sealed plastic bag at −80 °C.
4. Pulverize the leaves by squeezing them in the frozen form. Homogenize the pulverized leaves in 250 mL of prechilled extraction buffer solution with a blender.
5. Collect the crude virus solution by filtering the homogenate through four layers of cheesecloth. Squeeze the cheesecloth to collect as much filtrate as possible.
6. Load the filtrate in centrifuge tubes. Balance the tubes with ultrapure water prior to centrifugation. Centrifuge at 10,500 rpm (11,800 × *g*) for 36 min at 4 °C with Fiberlite F10-4× 1000 LEX rotor.
7. Collect the supernatant via filtration with four layers of cheesecloth. Record the volume of the filtrate (*x* mL where *x* is the volume obtained).
8. To the filtrate, add ½(*x*) mL of chloroform and ½(*x*) mL of n-butanol to make a volume ratio ($V_{Aqueous}$: $V_{Organic}$) of 1:1. Stir the mixture for 30 min at 4 °C (adjust proper stirring speed to avoid foaming). Fill the centrifuge tubes again with the homogenized mixture and carefully balance the tubes before centrifugation.
9. Centrifuge at 6000 rpm (3871 × *g*) for 18 min at 4 °C with F10–4× 1000 LEX rotor.
10. Collect the crude TMV solution (top layer) via careful decantation. Keep the solution at 4 °C or on ice. Record the weight ($m_{virus\ solution}$) and volume ($V_{virus\ solution}$) of the crude virus solution.
11. To the crude virus solution, add NaCl to 0.2 M ($m_{NaCl} = 0.2\ mol/L \times V_{virus\ solution} \times 55.44\ g/mol$), add PEG 8000 to 8% (w/w, $m_{PEG\ 8000} = m_{virus\ solution} \times 8\%$), add Triton X-100 surfactant to 1% (w/w, $m_{Triton\ x\text{-}100} = m_{virus\ solution} \times 1\%$). Stir at 4 °C for 30 min then store the solution for 1 h at the same temperature.
12. Load the solution into centrifuge tubes, carefully balancing the tubes with ultrapure water. Centrifuge at 10,500 rpm (11,800 × *g*) for 55 min at 4 °C with Fiberlite F10-4× 1000 LEX rotor.

13. Discard the supernatant and suspend the pellet in 20 mL phosphate buffer with the assistance of pipetting (*see* **Note 5**). Further homogenize the resuspension by gently stirring at 4 °C overnight.
14. To the centrifuge tube, add 60 mL of 40% sucrose solution. Carefully layer the suspended solution onto the top of the sucrose solution and fill the tube to the top. Two separate layers should be formed at this point.
15. Carefully balance the tubes with ultrapure water and centrifuge at 37,000 rpm (128,000 × g) for 160 min at 4 °C with Fiberlite F37 L-8× 100 rotor.
16. Discard the supernatant and suspend the pellet in 5–10 mL (*see* **Note 6**) of 0.01 M phosphate buffer overnight as described in **step 13**.
17. Split the suspended solution into 1.5 mL Eppendorf Microcentrifuge tubes. Centrifuge the solutions at 14,500 rpm (or 11,300 × *g*) for 15 min in Beckman Coulter Microfuge 16 centrifuge.
18. Collect the supernatant as the purified TMV solution. Store the TMV solution at 4 °C.
19. The concentration of TMV is determined by UV-Vis measurement on NanoDrop spectrophotometer. The absorbance at 260 nm is used to calculate the concentration with Beer's law ($A = \varepsilon lc$) and $\varepsilon = 3.0$ mL mg^{-1} cm^{-1}. The ratio of absorbance at 260 nm and 280 nm (A260/A280) should be around 1.2.
20. The purity of TMV solution is evaluated by investigating the absorbance at 260 nm when employing size exclusion chromatography with G3000SW$_{XL}$ column on an Agilent 1100 series HPLC system.

3.2 Desalination of TMV Solution

1. Flush the GE Health Care PD midi Trap G-25 column with 4 mL of ultrapure water four times to prepare it for use.
2. Add 1 mL of virus solution into the column and wait for the solution to load onto the column. The flow-through can be discarded.
3. Add 1.5 mL of ultrapure water to the column and collect the eluate as desalted virus solution (*see* **Note 7**).

3.3 Preparation of Thin ZIF-8 Layer on TMV (TZ-Thin)

1. In a 1.5-mL microcentrifuge tube, add desalted TMV solution containing 0.0625 mg virus particles (*see* **Note 8**), followed by addition of 500 μL of the 400 mM HMIM aqueous solution and 500 μL of the 20 mM $Zn(OAc)_2$ aqueous solution (*see* **Note 9**). The reaction mixture will become turbid immediately.

2. Shake the tube by hand (horizontally back and forth) for 20 s. At this point a whitish flocculate can be seen clearly in the reaction solution.
3. Set the tube on the bench under ambient conditions overnight (approx. 16 h). The flocculates will sediment but the liquid will remain turbid over this reaction time.
4. Centrifuge the reaction mixture at 2656 × *g* for 10 min to pellet the white sediment to the bottom of the tube.
5. Discard the clear supernatant gently with a pipette. Add 1000 μL of ultrapure water and suspend the flocculates via vortex.
6. Repeat **steps 4** and **5** to wash the product again. Finally, a suspension of TZ-thin in ultrapure water is obtained.

3.4 Preparation of Thick ZIF-8 Layer on TMV (TZ-Thick)

1. In a 20-mL scintillation vial, add desalted TMV solution that contains 0.111 mg virus particles (*see* **Note 8**), followed by three separate aliquots (3×) of 1000 μL of the 400 mM HMIM aqueous solution and three separate aliquots (3×) of 500 μL of the 20 mM $Zn(OAc)_2$ aqueous solution (*see* **Note 9**). The reaction mixture will turn turbid immediately.
2. Shake the vial (horizontally back and forth) for 20 s by hand and whitish flocculates should be visible at the bottom of the vial (*see* **Note 10**).
3. Set the vial on the bench under ambient conditions overnight (approx. 16 h). The flocculates sediment to the bottom but the liquid will stay turbid over the reaction time.
4. Transfer the reaction mixture into three 1.5-mL microcentrifuge tubes then centrifuge at 2656 × *g* for 10 min to pellet the white sediment to the bottom of the tube.
5. Discard the clear supernatant gently with a pipette. Add 1000 μL of ultrapure water to suspend the flocculates via vortex.
6. Repeat **steps 4** and **5** to wash the product again. Finally, a suspension of TZ-thick in ultrapure water is obtained.

3.5 Recover Virus Particles Via Exfoliation of ZIF-8 Shell

For best results, use at least 1 mg of TMV for TZ-thin/TZ-thick preparation and exfoliation. The exfoliation procedure described below was based on TZ-thin that was prepared using 1 mg of TMV.

1. Centrifuge and wash the as-prepared TZ-thin/TZ-thick as previously described in Subheading 3.3 (**steps 4–6**).
2. Add 1.0 mL of 1.0 M EDTA aqueous solution to the precipitate after the second wash. Vortex the mixture for 30 s to obtain a good suspension. Leave it on the benchtop.
3. After about 2 h the ZIF-8 should fully dissolve in the EDTA solution.

4. Add the as-obtained clear solution (1000 μL) to a preflushed GE Health Care PD midi Trap G-25 column.
5. Add 1.5 mL ultrapure water to the column and collect the eluate.
6. The concentration of recovered TMV is determined by UV-Vis measurement on NanoDrop. The absorbance at 260 nm is used to calculate the concentration with Beer's law ($A = \varepsilon lc$) and $\varepsilon = 3.0$ mL mg^{-1} cm^{-1}. The ratio of absorbance at 260 nm and 280 nm (A260/A280) should be around 1.2.

3.6 Characterization

3.6.1 Scanning Electron Microcopy

1. Cut a silicon wafer into 3 mm × 5 mm chips. Clean the silicon chips in acetone with the assistance of sonication.
2. Place double-sided copper tape onto the SEM sample mount, then put the clean silicon chip on the copper tape.
3. Add 5 μL of TZ-thin/TZ-thick suspension onto silicon chip and wait for 20 s.
4. Remove the excess suspension with a piece of filter paper. Wait for 30 min to let sample air-dry.
5. Conduct SEM with a beam current of 2.5 kV and working distance of approximately 8 mm (Fig. 2a, c).

3.6.2 Transmission Electron Microscopy for TZ-Thin/TZ-Thick

1. Conduct glow-discharge for the FCF200-Cu TEM grids to prepare a clean and hydrophilic surface.
2. Add 5 μL of TZ-thin/TZ-thick suspension onto FCF200-Cu sample grid and wait for 30 s. Remove excess suspension by placing a piece of filter paper at the edge of the droplet.
3. Place the loaded TEM grid into the sample holder and conduct TEM characterization at 120 kV (Fig. 2b, d).

3.6.3 Transmission Electron Microscopy for Exfoliated TZ-Thin/TZ-Thick

1. Conduct glow-discharge for the TEM grids.
2. Add 5 μL of exfoliated TZ-thin/TZ-thick suspension onto FCF200-Cu sample grid and wait for 30 s. Remove excess suspension by placing a piece of filter paper at the edge of the droplet.
3. Add 5 μL of 2% uranyl acetate aqueous solution and wait for 30 s. Remove excess suspension by placing a piece of filter paper to the edge of the droplet. Discard filter paper in waste designated for heavy metals.
4. Place the loaded TEM grid into the sample holder and conduct TEM characterization at 120 kV (Fig. 3).

3.6.4 Powder X-Ray Diffraction (PXRD)

1. Wash freshly prepared TZ-thin/TZ-thick with methanol two times (the volume of methanol equals to the total volume of precursor solutions). Dry the product under high vacuum overnight (approx. 16 h). An amount of 20 mg of dried material should be used for PXRD characterization.

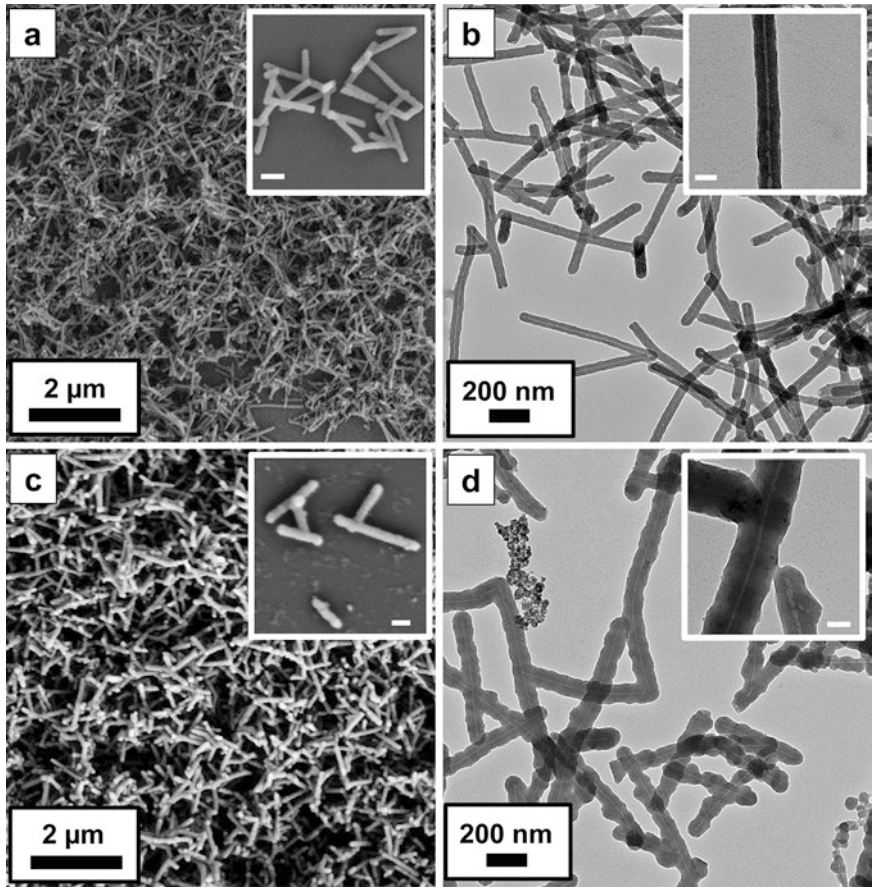

Fig. 2 Characterization of as-prepared TZ-thin in (**a**) SEM and (**b**) TEM; as-prepared TZ-thick in (**c**) SEM and (**d**) TEM. The inserted scale bar is 200 nm in (**a**) and (**c**); 50 nm in (**b**) and (**d**). Figure is reproduced from Ref. [20] with permission from Wiley

2. Grind the dry particulates prior to the PXRD measurement to obtain a fine powder without large granulated particles.
3. Conduct 2θ measurement from 5° to 80° with 0.02° step size (Fig. 4).

3.6.5 N_2 Sorption Analysis

1. Measure and record the mass of an empty sample container.
2. Load the TZ-thin/TZ-thick dry powder and measure the total mass. The loaded amount of sample should be at least 20 mg.
3. Heat the sample at 120 °C under vacuum overnight (approx. 16 h) to activate ZIF-8.
4. Measure the total mass again after heating and use that value to calculate sample mass.

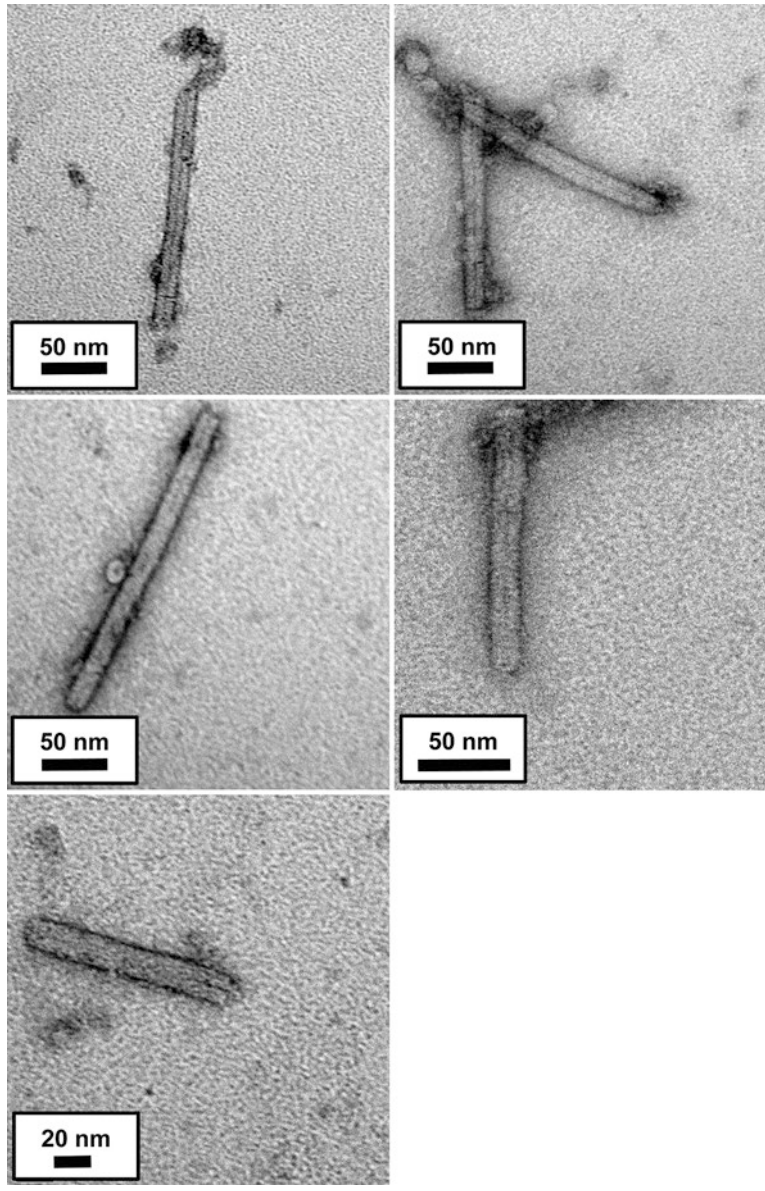

Fig. 3 TEM characterization of exfoliated TZ-thick. The sample is stained by 2% uranyl acetate solution. Figure is reproduced from Ref. [20] with permission from Wiley

5. Conduct N_2 sorption analysis at 77 K in a liquid nitrogen bath.
6. Calculate Brunauer–Emmett–Teller (BET) surface area with P/P_0 range of 0.01–0.1 (Fig. 5).

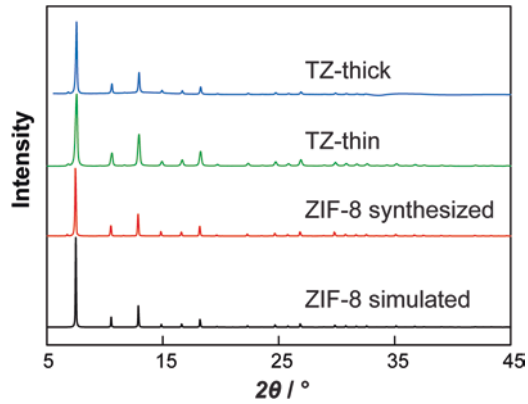

Fig. 4 PXRD pattern of as-synthesized ZIF-8, TZ-thin and TZ-thick. The simulated PXRD pattern of ZIF-8 is shown at the bottom. Figure is reproduced from Ref. [20] with permission from Wiley

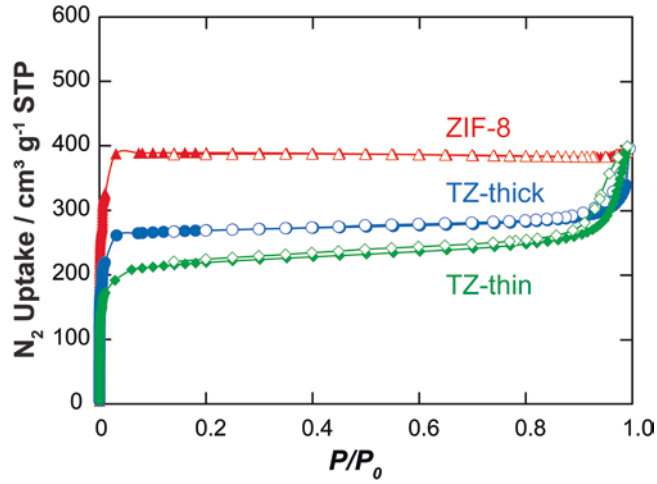

Fig. 5 N_2 sorption isotherm of ZIF-8, TZ-thin, and TZ-thick. The BET surface area of ZIF-8, TZ-thick, and TZ-thin is calculated as 1537, 1053, and 847 $m^2\ g^{-1}$, respectively. Figure is reproduced from Ref. [20] with permission from Wiley

4 Notes

1. To obtain optimal results, all precursor solutions should be freshly prepared.
2. The container should be covered with aluminum foil to avoid precipitation caused by light exposure. Disposal and handling should always be done in accordance with law.
3. It usually takes 8–10 weeks from seeding to being ready for infection.
4. The silicon carbide acts as an abrasive to break the leaf tissue. The particle size of silicon carbide powder is very small. Appropriate personal protective equipment should be used at

all times when handling it. Special care needs to be taken to avoid inhalation and contact with eyes. Gloves should be worn at all times. If too much silicon carbide is on the leaf, slightly tap the leaf to get rid of the excess powder; do not blow as this will aerosolize the powder. In general, the hand used for rubbing leaves should not touch the silicon carbide or the pipette. It is recommended that two people work together so that one can sprinkle the silicon carbide and add infection solution while the other rubs the leaves.

5. To resuspend the pellet, gently agitate the solution via suction and injection with a pipette. It is helpful if the stream of solution is pointed directly at the pellet to facilitate the resuspension.
6. We prefer to add the least amount of phosphate buffer as possible to make a concentrated virus solution, since it will take extra steps and produce more waste to a make a more concentrated virus solution later.
7. The eluate is collected from the first drop. UV-Vis can be used to check fractions to make sure that recovery is complete.
8. We recommend using TMV solutions with concentrations between 2 and 9 mg/mL and thus the volume of solution should fall within that range. The goal is to maintain the concentration of ZIF-8 precursors as the added volume of TMV solution is negligible and typically on the order of 10–30 μL in total when using TMV solutions between 2 and 9 mg/mL. If the TMV solution is too dilute then a large volume of TMV solution is needed, which could cause a significant change in the concentration of precursor solution. The concentration of precursor solution is crucial to the success of making TZ-thin/TZ-thick.
9. It is important to follow the order of addition as written. If the $Zn(OAc)_2$ solution is added first, discrete rod-like nanoparticles will not be obtained. Moreover, the $Zn(OAc)_2$ solution should be added as quickly as possible after addition of the HMIM solution. Delaying the addition of metal precursor will produce rods with an incorrect morphology.
10. The reaction mixture should be much thicker than the resulting suspension of TZ-thin.

Acknowledgments

We are grateful to the generous assistance we received from Professor Gerald Stubbs and Professor Nicole F. Steinmetz when starting our group for providing *N. benthamiana* seeds and TMV stock solutions. We would have been unable to conduct this research without the assistance they provided when others declined.

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