Protocol

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Publisher’s note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.
Protocol

Protocol for fabrication and characterization of Fe-SAC@COF for electrocatalytic oxygen evolution reaction

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SUMMARY

Single-atom catalysts (SACs) have promising atomic utilization efficiency and catalytic activity, but their construction with a specific coordination mode remains challenging. This protocol describes the synthesis of a coordinated Fe-SAC@COF for boosted electrocatalytic oxygen evolution reaction (OER). We also detail the steps for single iron atoms confinement and characterization of the COF and Fe-SAC@COF with X-ray diffraction and transmission electron microscopy technique. For complete details on the use and execution of this protocol, please refer to Wang et al. (2022).

BEFORE YOU BEGIN

Owing to unique electronic structures and maximized atom utilization, single-atom catalysts (SACs) exhibit intriguing OER performance. Covalent organic frameworks (COFs) with well-defined and tailorable structures could provide a platform for the variable coordination of isolated metal atoms by the confinement effect and coordination interaction with specified atoms of the organic skeleton. The electronic structure of the metal atom in SACs is crucial to catalytic activity. By tuning the backbones and functional groups as well as the coordinating geometry, unusually coordinated SACs could be constructed, which remains a great challenge in practice. Typically, Iridium (Ir) and Rhodium (Rh) constitute the most important class of OER electrocatalysts and has been known as the most efficient catalyst. However, despite their excellent catalytic activity, extensive commercialization is largely hindered by high cost and poor stability. Therefore, transitional metal catalysts Fe, Co, Ni) have attracted immense attention for OER owing to their unique electronic structures. Iron, an abundant and environmentally friendly element, has attracted research interest. In this protocol, we present the synthesis of Fe-SAC, which is confined in a covalent organic framework (Fe-SAC@COF) via low-temperature operation. The protocol can be applied for the synthesis of other single metal atoms (e.g., Ni) in COF skeletons with a specific coordination configuration.

In this protocol, we report a synthetic strategy of Fe-SAC@COF. At first, the COF is synthesized from 1,3,5-triformylphloroglucinol (Tp) and 4,4',4''-(1,3,5-triazine-2,4,6-triy)trianiline (Tta), then the corresponding Fe-SAC@COF is prepared via the coordination of Fe atom with COF via a low-temperature method.

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Chemicals, peptides, and recombinant proteins | | |
| Iron acetate (Fe(OAc)₂), GR | Sigma-Aldrich | CAS: 3094-87-9 |
| Ammonium hydroxide (NH₄OH, 30%), AR | Sigma-Aldrich | CAS: 1336-21-6 |
| Hydrochloric Acid (HCl, 36%), AR | ChemSupply | CAS: 7647-01-0 |

(Continued on next page)
### MATERIALS AND EQUIPMENT

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Potassium hydroxide (KOH), AR | Macklin | CAS: 1310-58-3 |
| 1,3,5-triformylphloroglucinol (Tp) | Jilin Chinese Academy of Sciences - Yanshen Technology | CAS: 34374-88-4 |
| 4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline (Tta) | Jilin Chinese Academy of Sciences - Yanshen Technology | CAS: 4544-47-9 |
| 1,4-dioxane, AR | Aladdin | CAS:123-91-1 |
| Mesitylene, AR | Aladdin | CAS:108-67-8 |
| Acetic acid, AR | Aladdin | CAS:64-19-7 |
| Acetone, AR | Aladdin | CAS:67-64-1 |
| Ethanol, AR | Aladdin | CAS:64-17-5 |
| Tetrahydrofuran, AR | Aladdin | CAS:109-99-9 |
| Nafion, AR | DuPont | DS20 |
| Hg/HgO electrode | Aida Hengsheng | R0501 |
| Platinum counter electrode | Aida Hengsheng | Pt017 |
| Nickel foam (NF) | Beijing InnoChem Science & Technology Co., Ltd. | CAS:7440-02-0 |
| Low-temperature constant temperature stirring reaction bath | Zhengzhou Ketai Laboratory Equipment Co., Ltd. | DHJK-8002 |
| Vacuum pump | Qingdao Lanten Science and Education Equipment Co., Ltd. | RS-2 |
| Flask clamps | N/A | N/A |
| Siderocradle | N/A | N/A |
| Aberration-corrected HAADF-STEM | www.shiyanjia.com | FEI Themis Z microscope |
| Electrochemical working station | Wuhan Corrtest Instruments Corp., LTD. | Bipotentiostat model: CS2350 |

#### Stock solution of dilute acetic acid solution (storage: 25°C)

| Reagent | Final concentration | Amount |
|---------|---------------------|--------|
| Concentrated acetic acid (17.5 M) | 6 M | 34.2 mL |
| Deionized water | N/A | 65.8 mL |
| Total | N/A | 100 mL |

Note: The 6 M HOAc can be stored at 25°C for one month.

#### Stock solution of dilute hydrochloric acid (storage: 25°C)

| Reagent | Final concentration | Amount |
|---------|---------------------|--------|
| Concentrated hydrochloric acid (12 M) | 3 M | 7.5 mL |
| Deionized water | N/A | 22.5 mL |
| Total | N/A | 30 mL |

Note: The 3 M HCl can be stored at 25°C for one month.

### STEP-BY-STEP METHOD DETAILS

**Synthesis of (1,3,5-triformylphloroglucinol)-(4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline) COF (Tp-Tta COF)**

- **Timing**: ~30 min, 3 days reaction, 1 h washing, 12 h dry
Synthesize Tp-Tta COF through the Schiff-base condensation reaction under solvothermal conditions by co-condensation of 1,3,5-triformylphloroglucinol (Tp) and 4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline (Tta) in the presence of the acetic acid catalyst, as shown in Figures 1 and 2 (Daugherty et al., 2019).

1. Measure 1,3,5-triformylphloroglucinol (Tp) (0.3 mmol, 63.4 mg) and 4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline (Tta) (0.3 mmol, 106.3 mg) by a precision balance and load into a Pyrex tube (volume: ca. 15 mL with a body length of 17 cm and a neck length of 9 cm) (Figure 1C).

   Note: When putting ligands into the Pyrex tube, the tube can be secured with a tube holder to prevent tipping.

2. Inject 3 mL mixture solution with 1.5 mL 1, 4-dioxane and 1.5 mL mesitylene into the mixture. After sonicating for 5 min, add 0.3 mL aqueous acetic acid (6 M), and sonicate the mixture for 10 min to form a homogeneous dispersion (Figure 2C).

3. Remove the gas from the solution by three freeze-pump-thaw cycles.

4. Flash-frozen the Pyrex tube in a liquid nitrogen bath, evacuate to an internal pressure of ca. 19 mbar using a vacuum pump, and flame-seal, which shortens the total length by ca. 5 cm (Figure 2D).

   △ CRITICAL: When flame-sealing, the operator should wear protective masks and not get too close to the equipment to avoid burns and injuries.

5. Upon thaw to room temperature, place the Pyrex tube in an oven at 120 °C for 3 days and then allow it to cool down to room temperature.

6. Collect precipitate by washing and centrifugation with tetrahydrofuran (THF, 3 × 40 mL) and acetone (3 × 40 mL) at 1760 g for 6 min to obtain a clear solution.

   △ CRITICAL: When washing the precipitate, if you are worried about the high speed, you can appropriately reduce the speed and increase the washing times. At the same time, during centrifugation, the cover of the centrifugal tube should be tightened to avoid spilling the solution and causing damage.

7. Collect the COF powder and dry it at 60 °C under vacuum for 12 h.

**Confine iron single atoms in the Tp-Tta COF (Fe-SAC@COF)**

○ Timing: ~1 h, 16+ h stirring at ~60 °C, 12 h dry
Synthesize the Fe-SAC@COF catalyst at low temperature to prevent the atom from aggregation (Figure 3) (Huang et al., 2019).

8. Mix deionized water and absolute ethanol with a volumetric proportion of 1 : 9 and ultrasonicate for 30 min to obtain ultralow-temperature solution as a solvent for a –60°C low-temperature reaction.

9. Add 5 mg Fe(OAc)$_2$ and 10 mL ultralow-temperature solution to a 50 mL flask.

   **Note:** Experimental technique is critical to the uniformity and content of a single atom. 5 mg Fe(OAc)$_2$ should be added to the flask first. After that, the solution is slowly injected using a 10-mL injector along the sides of the flask to ensure that Fe(OAc)$_2$ is fully incorporated into the solution.

10. Disperse the solution in an ice/water mixture to form a highly uniform for 30 min (Figure 4A).

   △ **CRITICAL:** It is recommended that the sonicated process has to be in an ice/water mixture. During the ultrasonic process, when the water temperature exceeds 25°C, the mixture of ice water needs to be continuously added to the ultrasonic cleaner.
11. Fix the flask with a clamp, immerse it into the low-temperature container and stir for 2 h, and keep the temperature at –60°C (Figure 4B).

12. Add 50 mg of COF powder to the above solution and stir for 12 h.

13. Add 0.05 mol NH$_3$·H$_2$O (1.75 g) and 0.5 mmol KOH powder (28 mg) into the above COF solution and mix for 2 h at –60°C.

△ CRITICAL: NH$_3$·H$_2$O alkaline solution should be cooled down to –60°C in advance under stirring and held for 30 min.

14. This was followed by washing with methanol (3 × 30 mL) until the solution is clear and collected by vacuum filtration (-0.1 MPa), and naturally dry at 25°C to obtain Fe-SAC@COF catalyst.

△ CRITICAL: Methanol has a low boiling point to remove organic solvent and uncoordinated metal. As alternative solvent, ethanol and water can be used to wash the Fe-SAC@COF catalyst.

Pausing Point: Store the obtained Fe-SAC@COF in a vial, you can use parafilm to avoid its interaction with atmosphere, keep it at a temperature range between 20°C–30°C.
Characterizations of COF and Fe-SAC@COF

Timing: ~10 min PXRD, ~3 days BET surface area, ~2 h HAADF-STEM

To evaluate and understand the structure properties of Fe-SAC@COF, X-ray diffraction (PXRD), Brunauer-Emmett-Teller (BET) surface area, and high-angle annular dark field-scanning transmission electron microscopy (HAADF-STEM) image are important.

15. After the successful preparation of COF and Fe-SAC@COF, characterize catalyst by powder X-ray diffraction (PXRD), Brunauer-Emmett-Teller (BET) surface area, and high-angle annular dark field-scanning transmission electron microscopy (HAADF-STEM) image (Figures 5 and 6).

Preparation of the working electrode

Timing: ~1 h washing nickel foam, ~1 h electrode preparation
Washing the nickel foam and coating the catalyst ink onto the nickel foam to obtain working electrode.

16. Soak a piece of nickel foam (NF, 1 cm × 1.5 cm) in diluted HCl aqueous solution (3 M) for 1 h to remove the oxidation layer on the surface, then clean with deionized water (3 × 30 mL) and ethanol (3 × 30 mL), respectively.
17. Add 5 mg of Fe-SAC@COF catalyst into a 1 mL mixture solvent containing ethanol and 5% Nafion (v/v=9/1) and sonicate for 30 min to form a homogeneous ink.
18. Drop 200 μL ink uniformly onto the NF within the area of 1 cm × 1 cm.
19. Dry the NF with catalyst ink by an infrared lamp for 20 min.

**Electrochemical measurement**

© Timing: ~30 min per catalyst

In this protocol, the electrocatalytic OER performance has been evaluated in a conventional three-electrode system using a workstation. Use Pt foil and Hg/HgO as the counter electrode and the reference electrode, respectively. Use 1.0 M KOH solution as the electrolyte, as shown in Figure 7.

20. Add 7 mg of KOH pellets into 125 mL of DI water to make 1.0 M KOH electrolyte under continuous stirring.

**Note:** The KOH solution should be cooled down before each test.

⚠️ CRITICAL: KOH is skin-irritant and when used, protective gear needs to be worn to avoid injury.

21. Pour 50 mL of electrolyte into the cell.
22. Use Fe-SAC@COF-coated NF, Pt foil, and Hg/HgO electrode as the working electrode, counter electrode, and reference electrode in the three-electrode measurement, respectively.
23. Activate the working electrode via cyclic voltammetry (CV) scanned from 0–0.8 V vs. Hg/HgO at a sweep rate of 50 mV s⁻¹ for at least 20 cycles.
24. Adopt linear sweep voltammetry (LSV) from 0–1 V vs. Hg/HgO at a sweep rate of 5 mV s⁻¹ to evaluate the catalytic OER performance.
25. Take out all electrodes and wash with DI water.
CRITICAL: Measure LSV following activation when the CV reaches stable curves.

EXPECTED OUTCOMES

This protocol provides a method for the preparation of single atoms for oxygen evolution reaction catalysis. In this regard, a Fe single-atom specie was confined in a two-dimensional covalent organic framework (COF) at low temperature. The obtained Fe-SAC@COF constitutes an unusual Fe-NO coordination in the skeleton.

For electrochemical OER catalysis, use the as-prepared Fe-SAC@COF as the working electrode. It can be observed that bare Ni foam showed low OER catalytic current density suggesting a weak and negligible activity for OER. On the contrary, the Fe-SAC@COF shows a record-low overpotential (290 mV) and Tafel slope (40 mV dec$^{-1}$) (Figure 8). This work provides a fabrication strategy toward novel coordinated SACs by prudent COF confinement for advanced electrocatalysis.

LIMITATIONS

The main limitation of this protocol is the preparation of the working electrode, we directly drop the ink of the catalyst onto a Ni foam, resulting in poor catalyst-support contact. Therefore, it is highly necessary to grow the catalyst in situ on the backbone of the NF, which can enhance the catalyst-support contact for more efficient transport of charge carriers and improve the adhesion against bubbling.

TROUBLESHOOTING

Problem 1
The crystallinity of the obtained COF may not be high (steps 1 and 2).
Potential solution
If the COF shows poor crystallinity, one possible solution is to tune the number of solvents.

Problem 2
Metal atoms could aggregate to form nanoparticles (steps 9 and 10).

Potential solution
Keep a constant and low temperature during sonication of the Fe(OAc)$_2$ solution, and increase sonication time to ensure the metal atoms are highly fragmented.

Problem 3
The catalyst ink may leak from the nickel foam during coating (step 19).

Potential solution
Coat the catalyst ink separately, and wait for drying before the next coating.

Problem 4
The LSV curve may vary due to the interruption of soluble air in the electrolyte and insufficient activation (step 24).

Potential solution
The electrolyte should be pre-saturated with Ar gas for at least 30 min. The working electrode needs to be activated by CV cycling until the last two curves are overlapped.

Problem 5
The detected BET surface area may not be accurate for the SAC@COF samples (step 15).

Potential solution
To get a reliable BET surface area, take at least 60 mg of the sample for detection.

RESOURCE AVAILABILITY
Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hao Wu (haowu2020@sdu.edu.cn).

Materials availability
This protocol did not generate new unique reagents.

Data and code availability
The published article includes all datasets/codes generated or analyzed during this study.

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AUTHOR CONTRIBUTIONS
X.W. drafted the manuscript. X.W. and W.Z. performed experiments and did analysis. H.W. edited the writing. H.W. and W.-Q.D. planned and supervised the entire study.

DECLARATION OF INTERESTS
The authors declare no conflict of interest.
REFERENCES

Wang, X., Sun, L., Zhou, W., Yang, L., Ren, G., Wu, H., and Deng, W.-Q. (2022). Iron single-atom catalysts confined in covalent organic frameworks for efficient oxygen evolution reaction. Cell Rep. Phys. Sci. 3, 100804.

Daugherty, M.C., Vitaku, E., Li, R.L., Evans, A.M., Chavez, A.D., and Dichtel, W.R. (2019). Improved synthesis of b-ketoenamine-linked covalent organic frameworks via monomer exchange reactions. Chem. Commun. 55, 2680-2683.

Huang, K., Zhang, L., Xu, T., Wei, H., Zhang, R., Zhang, X., Ge, B., Lei, M., Ma, J.Y., Liu, L.M., and Wu, H. (2019). 60°C solution synthesis of atomically dispersed cobalt electrocatalyst with superior performance. Nat. Commun. 10, 606.

Chen, P.Z., Zhou, T.P., Xing, L.L., Xu, K., Tong, Y., Xie, H., Zhang, L.D., Yan, W.S., Chu, W.S., Wu, C.Z., and Xie, Y. (2017). Atomically dispersed iron-nitrogen species as electrocatalysts for bifunctional oxygen evolution and reduction reactions. Angew. Chem. Int. Ed. Engl. 56, 610-614.

Pan, Y., Liu, S.J., Sun, K.A., Chen, X., Wang, B., Wu, K.L., Cao, X., Cheong, W.C., Shen, R.A., Han, A.J., et al. (2018). A bimetallic Zn/Fe polyphthalocyanine-derived single-atom Fe-N4 catalytic site: a superior trifunctional catalyst for overall water splitting and Zn-air batteries. Angew. Chem. Int. Ed. Engl. 57, 8614-8618.

Zhang, J.T., Zhang, M., Zeng, Y., Chen, J.S., Qiu, L.X., Zhou, H., Sun, C.J., Yu, Y., Zhu, C., Zhu, Z.H., et al. (2019). Single Fe atom on hierarchically porous S, N-Co doped nanocarbon derived from porphyra enable boosted oxygen catalysis for rechargeable Zn-air batteries. Small 15, 1900307.

Lei, C.J., Chen, H.Q., Cao, J.H., Yang, J., Qiu, M., Xia, Y., Yuan, C., Yang, B., Li, Z.J., Zhang, X.W., et al. (2018). Fe-N4 sites embedded into carbon nanofiber integrated with electrochemically exfoliated graphene for oxygen evolution in acidic medium. Adv. Energy Mater. 8, 1801912.

Zeng, X.J., Shui, J.L., Liu, X.F., Liu, Q.T., Li, Y.C., Shang, J.X., Zheng, L.R., and Yu, R.H. (2018). Single-atom to single-atom grafting of Pt1 onto Fe-N4 Center: Pt1@Fe-N-C multifunctional electrocatalyst with significantly enhanced Properties. Adv. Energy Mater. 8, 1701345.

Ho, Y., Qiu, M., Kim, M.G., Liu, P., Nam, G., Zhang, T., Zhuang, X., Yang, B., Cho, J., Chen, M., et al. (2019). Atomically dispersed nickel-nitrogen-sulfur species anchored on porous carbon nanosheets for efficient water oxidation. Nat. Commun. 10, 1392.

Ma, L.T., Chen, S.M., Pei, Z.X., Huang, Y., Liang, G.J., Mo, F.N., Yang, Q., Su, J., Gao, Y.H., Zapien, J.A., and Zhu, C.Y. (2018). Single-site active iron-based bifunctional oxygen catalyst for a compressible and rechargeable zinc-air battery. ACS Nano 12, 1949-1958.