Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Urate oxidase-loaded in PCN-222(Fe) with peroxidase-like activity for colorimetric detection of uric acid

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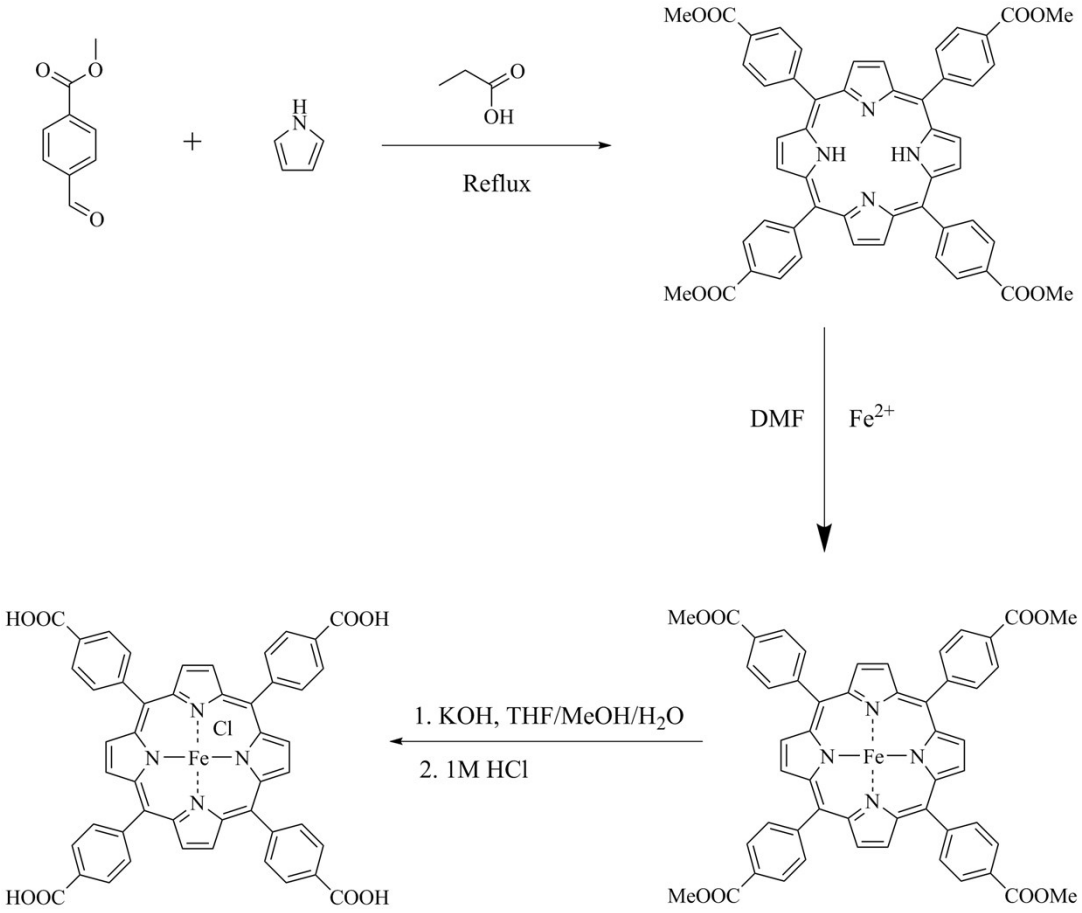
Experimental section

Synthesis of TPP-COOMe and Fe-TCPP

TPP-COOMe [5,10,15,20-tetrakis(4-methoxycarbonylphenyl) porphyrin] and Fe-TCPP ligand were synthesized according to the previous procedure with slight modification (*J. Porous Mater.*, 2019, 26: 1507), as shown in Scheme S1. Briefly, propionic acid (100 mL), methyl 4-formylbenzoate (6.3 g) and pyrrole (3.0 g) were first mixed in a 250-mL three necked flask, and the mixture was refluxed for 12 h in the dark. After cooling to room temperature, purple crystals (TPP-COOMe) were collected by suction filtration. For the synthesis of Fe-TCPP, TPP-COOMe (0.854 g) and $FeCl_2 \cdot 4H_2O$ (2.5 g) were dissolved in 100 mL DMF, and the solution was refluxed for 6 h in the dark. Then, 150 mL distilled water was added into the mixture after cooling to room temperature. Following the stirring for 1 h, the obtained precipitate was filtered and washed twice with distilled water. The dark green crystals were then dissolved in $CHCl_3$ and washed with distilled water three times, and the organic layer was dried over anhydrous magnesium sulfate and evaporated to obtain crystals. The crystals (0.75 g) were dissolved in a mixture of methanol (25 mL), tetrahydrofuran (THF, 25 mL) and KOH solution (2 mM, 25 mL), and the sample was then refluxed in the dark for 12 h. THF and methanol were subsequently removed by evaporation in vacuo, and 1 M HCl were added to acidify the solution until no further precipitate was detected. Finally, Fe-TCPP was collected by filtration, washed with distilled water and dried in vacuo.



Scheme S1. Synthesis steps for TPP-COOMe and Fe-TCPP.

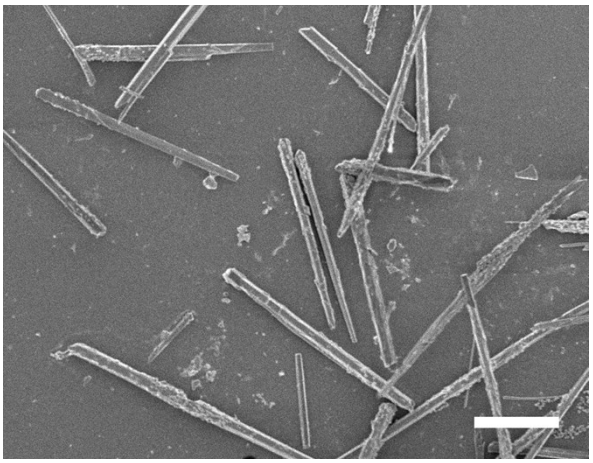


Figure S1. SEM image of UOx@PCN-222(Fe). Scale bar: 20 μm.

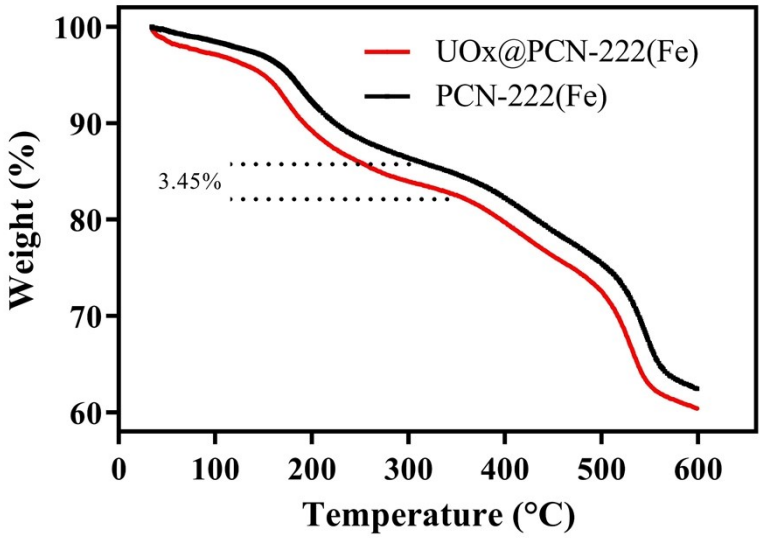


Figure S2. TGA curve of PCN-222(Fe) and UOx@PCN-222(Fe).

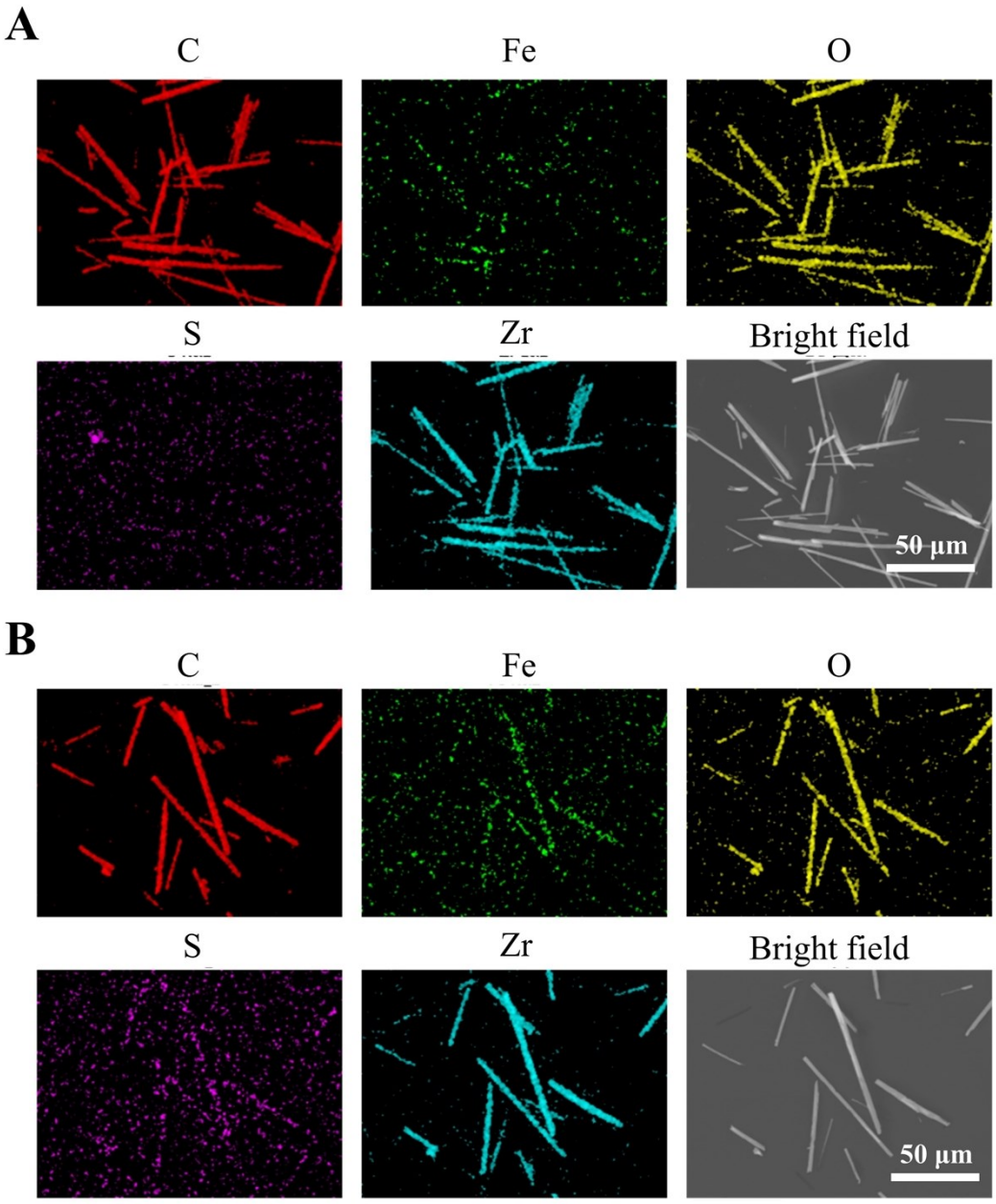


Figure S3. EDX mapping images of PCN-222(Fe) and UOx@PCN-222(Fe) for C, Fe, O, S and Zr elements.

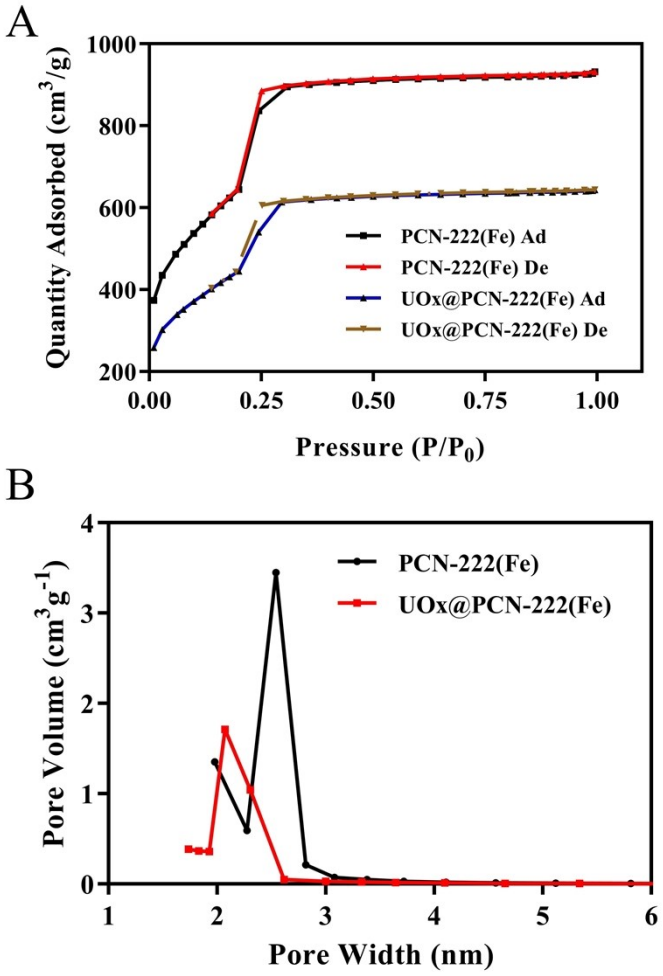


Figure S4. (A) N_2 adsorption/desorption isotherms of PCN-222(Fe) and UOx@PCN-222(Fe). (B) Pore size distribution of PCN-222(Fe) and UOx@PCN-222(Fe).

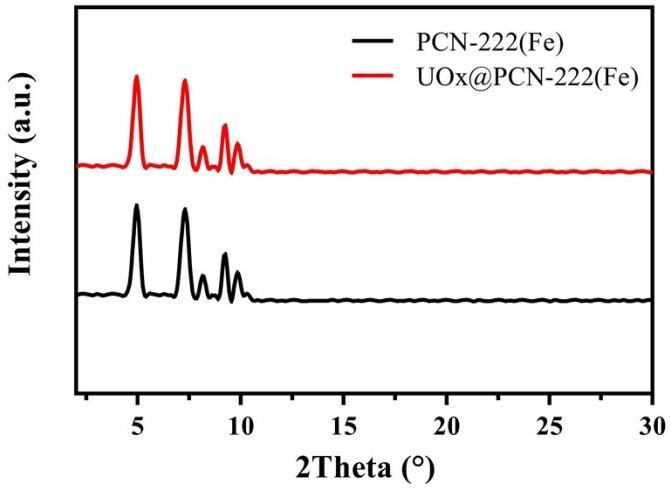


Figure S5. PXRD patterns of PCN-222(Fe) and UOx@PCN-222(Fe).

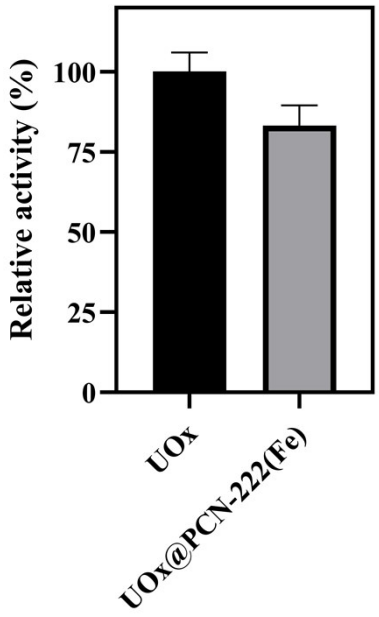


Figure S6. The comparison of enzymatic activity of free UOx and immobilized UOx in PCN-222(Fe), using Amplex Red uric acid/uricase assay kit.

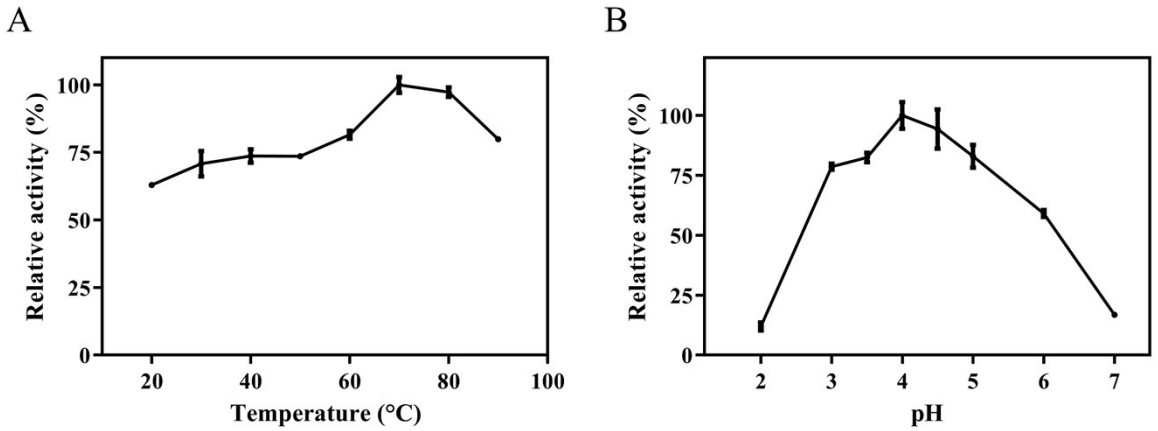


Figure S7. Effects of temperature (A) and pH (B) on the peroxidase activity of PCN-222(Fe). Experiments were performed in PBS (50 mM) using PCN-222(Fe) (20 μg/mL) with TMB (1.0 mM) and H_2O_2 (0.5 mM) as substrates. Data were presented as mean ± standard derivation of triplicate experiments.

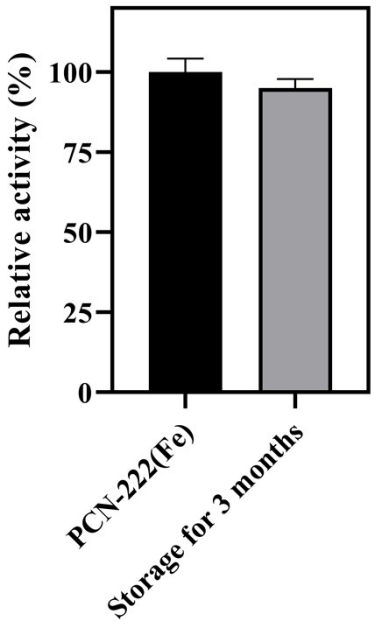


Figure S8. Residual enzymatic activity of PCN-222(Fe) after the storage for 3 months. Data were presented as mean ± standard derivation of triplicate experiments.

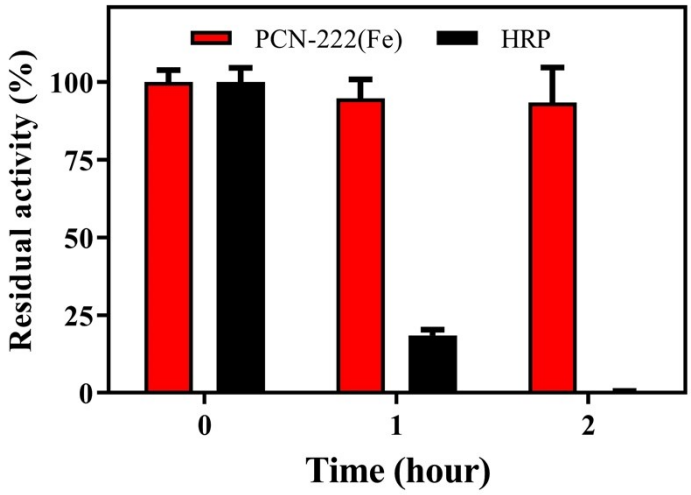


Figure S9. Residual enzymatic activity of PCN-222(Fe) and HRP after the incubation in PBS (50 mM, pH 2.0) for different time. Data were presented as mean ± standard derivation of triplicate experiments.

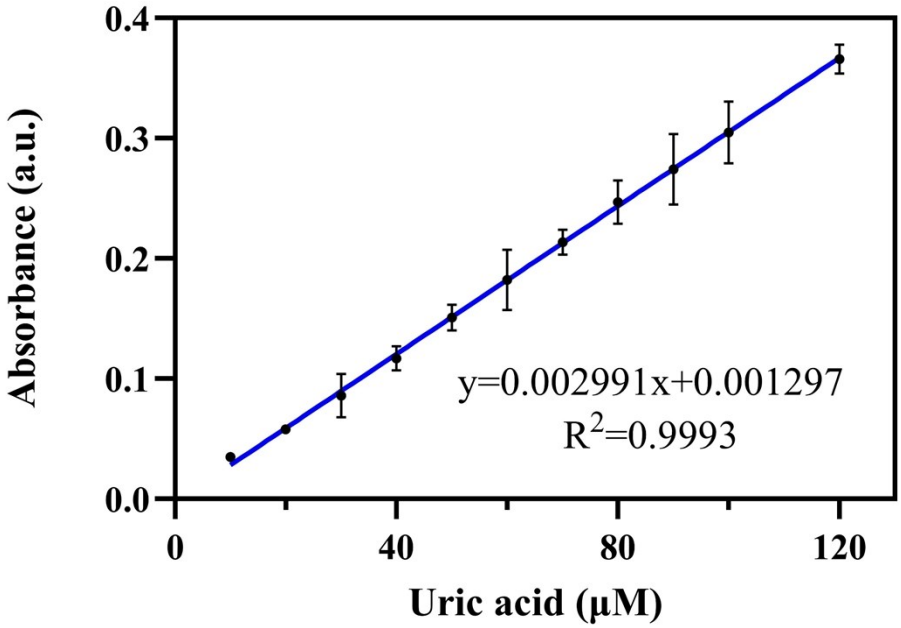


Figure S10. Standard curve of UA measurement from Amplex Red uric acid/uricase assay kit at a wavelength of 540 nm (UA concentration of 0-120 μM).

Table S1. BET surface area and pore size of PCN-222(Fe) and UOx@PCN-222(Fe).

Sample	BET surface area (m^2/g)	Pore size(nm)	Total pore volume (cm^3/g)
PCN-222(Fe)	2871.39	2.58	1.431
UOx@PCN-222(Fe)	1177.22	2.03	0.991

Table S2. Michaelis-Menten kinetic parameters of PCN-222(Fe) for substrates TMB and H_2O_2 and the comparison with other catalysts in the literature.

Catalysts	K_m (mM)		V_{max} (10^{-8} M s^{-1})	
	TMB	**H_2O_2**	**TMB**	**H_2O_2**
PCN-222(Fe)	1.766	0.504	6.099	4.343
HRP[1]	0.434	3.70	10.0	8.71
AgNC[2]	0.216	0.207	1.20	1.07
Hemin[3]	4.26	2.95	1.108	0.637
Fe_3O_4[4]	0.098	154.0	3.44	9.78
MoS_2 NPs[5]	4.55	0.019	3.62	0.244
Heme-ZrMOF[6]	0.41	0.40	30.10	4.69

Table S3. Comparison of different systems for UA detection.

Catalysts	Linear range (μM)	LOD (μM)	Detection time (min)
UOx@PCN-222(Fe)	10-800	3.5	25
CCA-YH[7]	5-800	0.577	55
MIL-53(Fe [8]	4.5-60	1.3	40
Au/Ag NCs[9]	5-50	5.1	90
MPADs[10]	100-1000	37	60
Keratin-NF@Ag_3PO_4[11]	3-100	0.94	8
g-C_3N_4[12]	10-100	8.9	60
UCNPs-MNPs[13]	10-100	2.86	60
GQD@Ag[14]	5-500	2	40
Fe@NCDs[15]	2-150	0.64	65

Table S4. Comparison of UA concentration values measured by the proposed method and other methods.

Serum	Biochemical analyzer (μM)	Determined by commercial kit (μM)	Determined by this work (μM)	Relative deviation (%)[a]
Serum 1	303	511.51 ± 25.08	283.91 ± 10.74	-6.30
Serum 2	266	392.46 ± 58.47	274.31 ± 19.80	+3.12
Serum 3	381	494.84 ± 28.87	367.84 ± 6.50	-3.45

[a]Relative deviation was calculated based on the difference between the UA concentration values of this work and biochemical analyzer.

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