Supporting Information

Room-Temperature Controllable Synthesis of Hierarchically Flower-like Hollow Covalent Organic Frameworks for Brain Natriuretic Peptide Enrichment

Wende Ma, Guorong Li, Chao Zhong, Yixin Yang, Qianqian Sun, Dan Ouyang, Wei Tong, Wenchang Tian, Lan Zhang, and Zian Lin*

Ministry of Education Key Laboratory of Analytical Science for Food Safety and Biology, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, College of Chemistry, Fuzhou University, Fuzhou, Fujian, 350116, China.

Corresponding author: Zian Lin

Postal address: College of Chemistry, Fuzhou University

2# Xueyuan Road, Qishan Campus, Fuzhou, Fujian 350116, P. R. China

Fax: +86-591-22866135

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EXPERIMENTAL SECTION

Materials. All chemicals and reagents were analytical grade or better and used without further purification. 1,3,5-Tris(4-aminophenyl)benzene (TPB), 2,5-divinylterephthalaldehyde (DVA), 2,5-dimethoxyterephthalaldehyde, 2,5-bis(2-propyn-1-yloxy)-1,4-benzenedicarboxaldehyde, 2,5-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)terephthalaldehyde, 2,5-dibutoxy-benzene-1,4-dicarbaldehyde, 2,5-bis-hexyloxy-benzene-1,4-dicarbaldehyde, 2,5-dioctoxyterephthalaldehyde, benzene-1,3,5-tricarbaldehyde, and 2,4,6-trimethoxy-1,3,5-benzenetricarbaldehyde were obtained from Jilin Chinese Academy of Sciences - Yanshen Technology Co., Ltd. Sodium tetrachloroaurate(III) dehydrate ($NaAuCl_4 \cdot 2H_2O$), sodium citrate (SC), tannic acid (TA), and potassium carbonate (K_2CO_3) were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). BNP (Mw = 3464.4) was obtained from Guotai Bio-technology Co. Ltd (Hefei, China). Deionized water (18.2 MΩ cm^{-1}) was produced by Milli-Q water purification system (Millipore, USA). Human serum samples were kindly donated by Fujian Provincial Governmental Hospital (Fuzhou, China). The protocol and written informed consent were approved by the Fujian Province Official Hospital Ethics Committee.

Instrumentation. Dynamic light scattering (DLS) data were recorded on NanoPlus3 nanoparticle size and zeta potential analyzer by using ethanol as the dispersion solution. Field emission scanning electron microscopies (SEM) images were obtained by a Verios G4 microscope equipment. Transmission Electron Microscopy (TEM) images were acquired on Hitachi HT7700 and FEI Talos F200S G2. Fourier transform infrared

(FT-IR) spectra of the solid samples were taken on a BD FACSCanto (TM) Ⅱ spectrometer. ^{13}C ssNMR spectrums were collected at a Bruker (11.7T/500MHz)/AVANCE Ⅲ 500 spectrometer. PXRD was carried out on a CEM DY5261/Xpert3 X-ray diffractometer, and the corresponding data were collected in the range of 2θ = 1.5-30° at a scan rate of 2° min^{-1}. N_2 adsorption-desorption isotherms were measured on a Micromeritics ASAP 2020 automatic volumetric instrument at 77 K. The samples were degassed at 150 °C for 8 h under vacuum before measurement. The surface areas were calculated from the adsorption data using Brunauer-Emmett-Teller (BET) model. The pore-size-distribution curve were obtained using the non-localized density functional theory (NLDFT). Thermogravimetric analysis (TGA) was carried out on a STA449C/6/G analyzer from 30 to 1000 °C under Ar_2 atmosphere with a ramp rate of 10 ºC min^{-1}.

Mass spectra were obtained by a MALDI-TOF/TOF MS system (Bruker Daltonics, Bremen, Germany) with a Nd: YAG laser at 355 nm, a repetition rate of 2000 Hz, and an acceleration voltage of 20 kV.

Synthesis of HFH-COF-1 with different sizes. TPB (0.04 mmol) and DVA (0.06 mmol) were individually dissolved in 5 mL of ACN solvent（92%） containing 1.9, 2.4, 2.9, and 3.4 M HAc, respectively. Subsequently, the mixture was sonicated for 1 min and incubated at room temperature for 72 h. The obtained yellow precipitates were collected by centrifugation at 10000 rpm for 3 min and washed with dry THF and ethanol for three times, respectively.

Synthesis of HFH-COF-1 in different proportion of ACN solvent. TPB (0.04 mmol) and DVA (0.06 mmol) were dissolved in ACN solvent (5 mL, containing 1.9 M HAc) with different proportion (100, 96, 92, 90, 88, 86, 84, 82, and 80%) and sonicated for 1 min and allowed to stand at room temperature for 72 h. The obtained yellow precipitates were collected by centrifugation at 10000 rpm for 3 min and washed with dry THF and ethanol for three times, respectively. Then the powders were dried under high vacuum for 24 h.

Synthesis of HFH-COF-1 at different time intervals. TPB (0.04 mmol) and DVA (0.06 mmol) were dissolved in 5 mL of ACN solvent（92%） containing 2.4 M HAc. Subsequently, the mixture was sonicated for 1 min and the individual sample was removed after specified time intervals (12 h, 24 h, 48 h, and 72 h). The precipitates were collected and dried in a similar condition as described before. The obtained materials were further characterized by TEM, SEM, BET, and PXRD.

Synthesis of other eight HFH-COFs. Synthesis of HFH-COF-2: TPB (0.04 mmol) and 2,5-dimethoxyterephthalaldehyde (0.06 mmol) were dissolved in 5 mL of ACN solvent（92%） containing 1.2 M HAc and the subsequent operation was same as mentioned above. Similarly, HFH-COF-3 was synthesized under conditions of TPB (0.04 mmol), 2,5-bis(2-propyn-1-yloxy)-1,4- benzenedicarboxaldehyde (0.06 mmol), 5 mL of ACN solvent（92%） containing 1.45 M HAc. HFH-COF-4 was synthesized under conditions of TPB (0.04 mmol), 2,5-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)terephthalaldehyde (0.06 mmol), 5 mL of ACN solvent（92%） containing 1.9 M HAc. HFH-COF-5 was synthesized under conditions of TPB (0.04 mmol), 2,5-

dibutoxy-benzene-1,4-dicarbaldehyde (0.06 mmol), 5 mL of ACN solvent（92%） containing 1.9 M HAc. HFH-COF-6 was synthesized under conditions of TPB (0.04 mmol), 2,5-bis-hexyloxy-benzene-1,4-dicarbaldehyde (0.06 mmol), 5 mL of ACN solvent（92%） containing 2.4 M HAc . HFH-COF-7 was synthesized under conditions of TPB (0.04 mmol), 2,5-dioctoxyterephthalaldehyde (0.06 mmol), 5 mL of ACN solvent（92%） containing 2.4 M HAc. HFH-COF-8 was synthesized under conditions of TPB (0.04 mmol), 2,4,6-trimethoxy-1,3,5-benzenetricarbaldehyde (0.04 mmol), 5 mL of ACN solvent（92%） containing 1.9 M HAc. HFH-COF-9 was synthesized under conditions of TPB (0.04 mmol), benzenedicarboxaldehyde (0.04 mmol), 5 mL of ACN solvent（92%） containing 2.4 M HAc.

Synthesis of Au nanoparticles. The products were prepared according to the previous literature with some modification. [1] A 75 mL of freshly prepared reducing solution of sodium citrate (SC, 48.75 mg) containing tannic acid (TA, 0.21 mg) and potassium carbonate (K_2CO_3, 10.5 mg) was heated with a heating mantle in a 150 mL round-bottom flask under vigorous stirring. When the temperature reached 70 °C, 1.0 mL of deionized water contained sodium tetrachloroaurate(III) dihydrate ($NaAuCl_4 \cdot 2H_2O$, 4.97mg) was injected. The solution was kept at 70 °C for 10 min to ensure complete reaction of the gold precursor.

Synthesis of HFH-COF-1@Au. The 20 mg of HFH-COF-1 was added into 4 mL of deionized water and the mixture was sonicated for 10 min to completely disperse the HFH-COF-1. Afterwards, 1.0 mL of deionized water contained 1.0 mg, 1.5 mg, 2.0 mg, and 2.5 mg $NaAuCl_4 \cdot 2H_2O$ was individually added to the above mixture solution and the tube were shaken vigorously for 30 min.

A 50 mL of freshly prepared reducing solution of sodium citrate (SC, 32.5 mg) containing tannic acid (TA, 0.14 mg) and potassium carbonate (K_2CO_3, 7.0 mg) was heated with a water bath in a 100 mL round-bottom flask under vigorous stirring. When the temperature reached 70 °C, the prepared above turbid solution was injected. The solution was kept at 70 °C under vigorous stirring for 10 min. The solid product was then filtered then washed three times water. Finally, the obtained product was dried in a vacuum oven at 60 °C for 24 h.

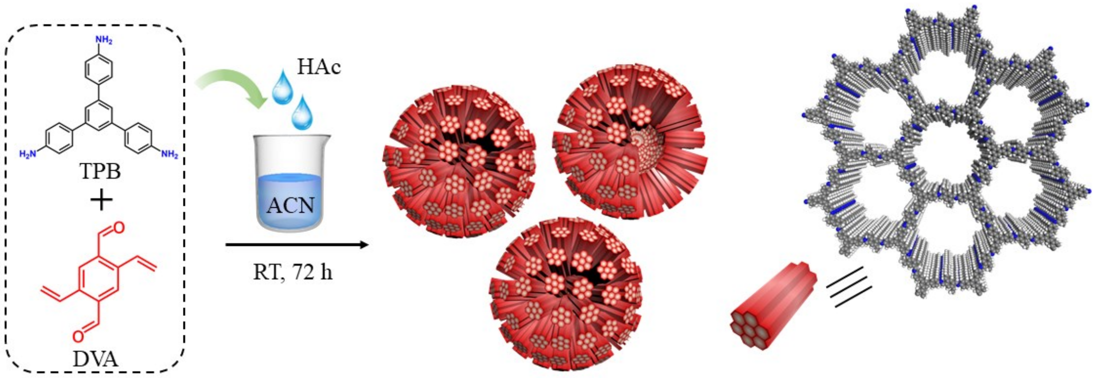
Adsorption performance evaluation of HFH-COF-1@Au. The adsorption experiments were carried out in a centrifuge tube under ambient conditions. Deionized water as the solvent of all sample solution. In the experiments, 1.0 mg of HFH-COF-1@Au (or Au nanoparticles or HFH-COF-1) were vortex-mixed with 1.0 mL of BNP solution (10 fmol/μL) for 15 min. Then the mixture solutions were centrifuged at 10000 rpm and the supernatants were collected. The BNP-captured HFH-COF-1@Au (or Au nanoparticles or HFH-COF-1) were eluted with 10 μL DHB (30 mg/mL, 50%ACN:49%H_2O:1%H_3PO_4) for 30 min. The supernatants and the eluates were analyzed by using MALDI-TOF-MS, respectively. The eluate of DHB (1 μL) was deposited as the matrix. The mass spectra were obtained in reflection positive mode.

To optimize the eluent conditions, 1.0 mg of HFH-COF-1@ Au was vortex-mixed with 1.0 mL of BNP solution (1.0 fmol/μL) for 15 min. Then the BNP-captured HFH-COF-1@Au was eluted with 5 μL, 10 μL, and 15 μL DHB (30 mg/mL, 50%ACN:49%H_2O:1%H_3PO_4) for 30 min, respectively, and the eluates were analyzed with MALDI-TOF-MS.

To evaluate the sensitivity, the standard BNP with different concentrations (1 fmol/μL, 0.1 fmol/μL, and 0.02 fmol/μL) were prepared with deionized water (1 mL). Then, the adsorption experiments were the same as above and the eluate was analyzed with MALDI-TOF-MS.

Enrichment of BNP from human serum. In the experiments, 10-fold diluted human serum (1 mL) were incubated with the HFH-COFs-1@Au (1.0 mg) for 15 min. After centrifugation, the supernatants were removed and washed with 50% ACN PBS buffer (20 mM) and water two times, respectively. The peptide-captured HFH-COFs-1@Au were eluted with 10 μL eluent (DHB). Finally, the eluate was analyzed by MALDI-TOF-MS analysis.

[1] J. Piella, N.G. Bastus and V. Puntes, *Chem. Mater*. 2016, **28**, 1066-1075.



Scheme S1. Room-temperature controllable synthesis of hierarchically flower-like hollow COFs (HFH-COF-1).

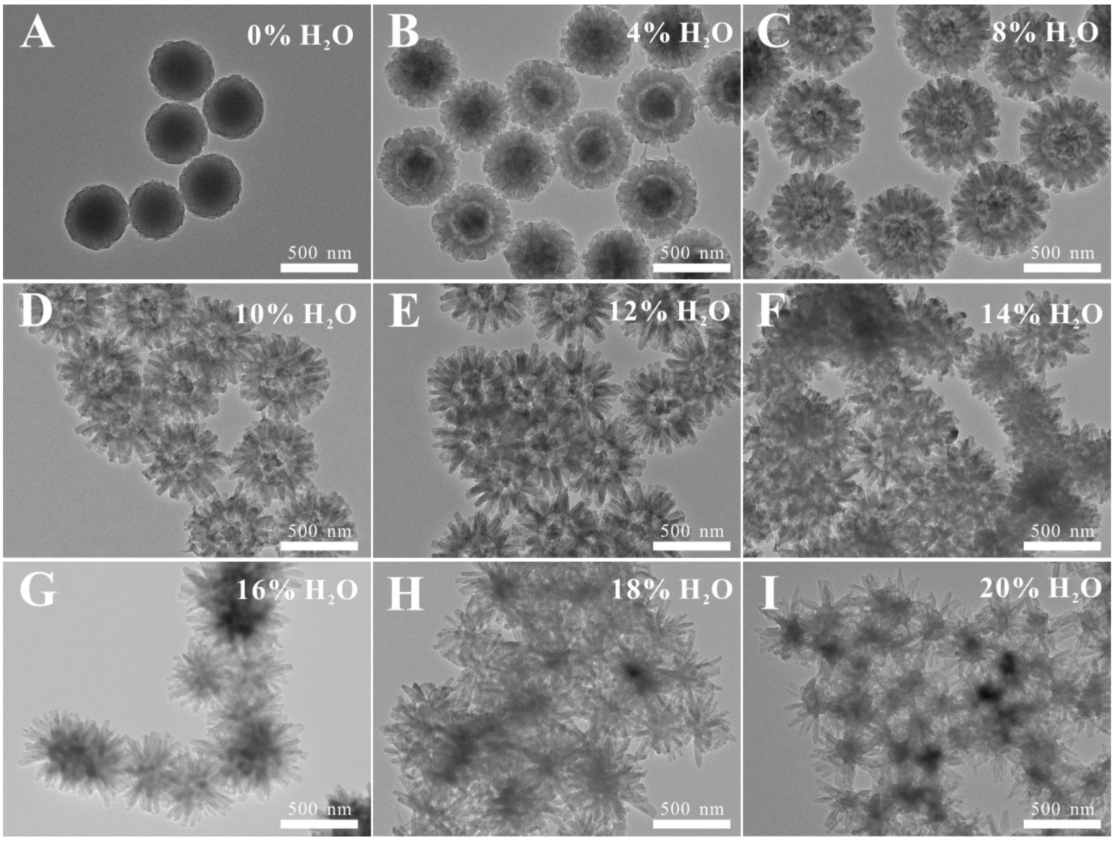


Figure S1. TEM images of COFs prepared with different proportion of H_2O in ACN solvent: (A) 0% H_2O, (B) 4% H_2O, (C) 8% H_2O, (D) 10% H_2O, (E) 12% H_2O, (F) 14% H_2O, (G) 16% H_2O, (H) 18% H_2O, (I) 20% H_2O, respectively.

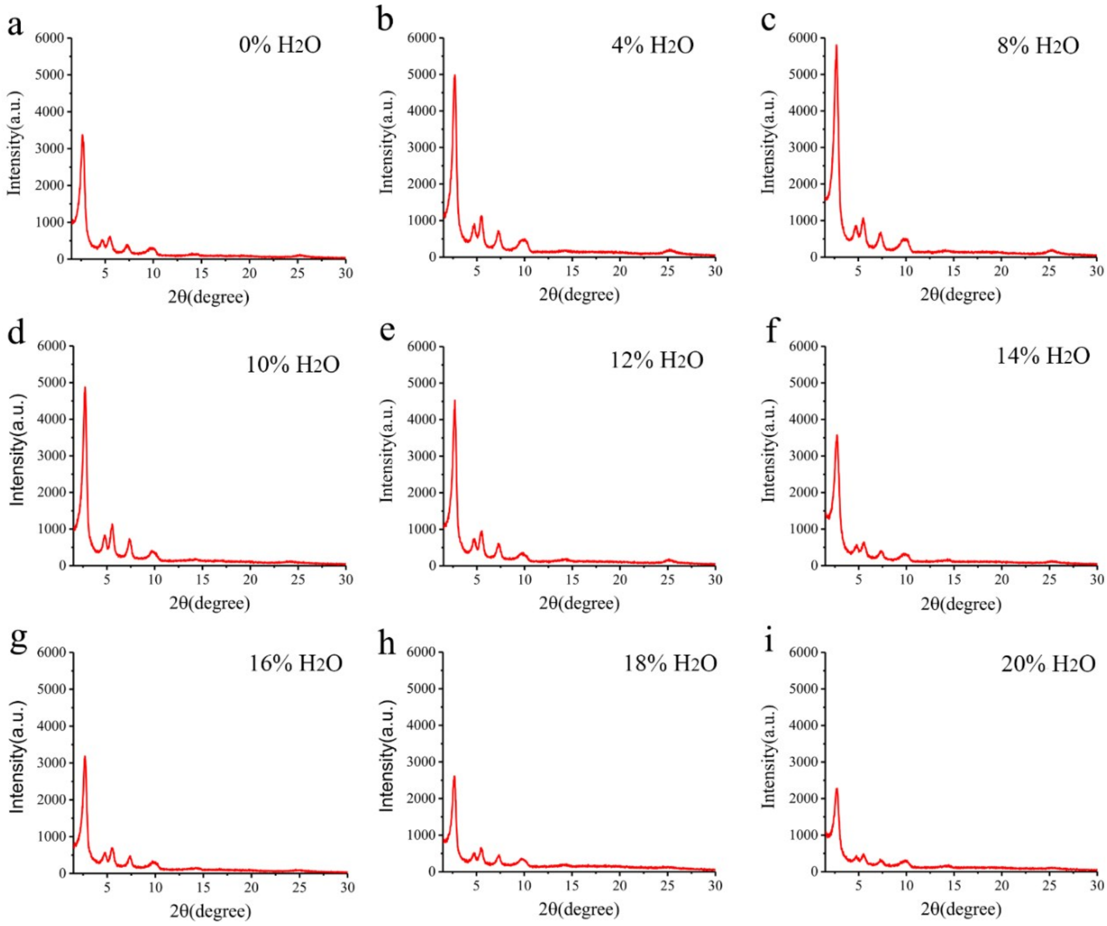


Figure S2. PXRD analysis of the COFs prepared in different proportion of H_2O in ACN solvent (5 mL): (a) 0% H_2O, (b) 4% H_2O, (c) 8% H_2O, (d) 10% H_2O, (e) 12% H_2O, (f) 14% H_2O, (g) 16% H_2O, (h) 18% H_2O, (i) 20% H_2O, respectively.

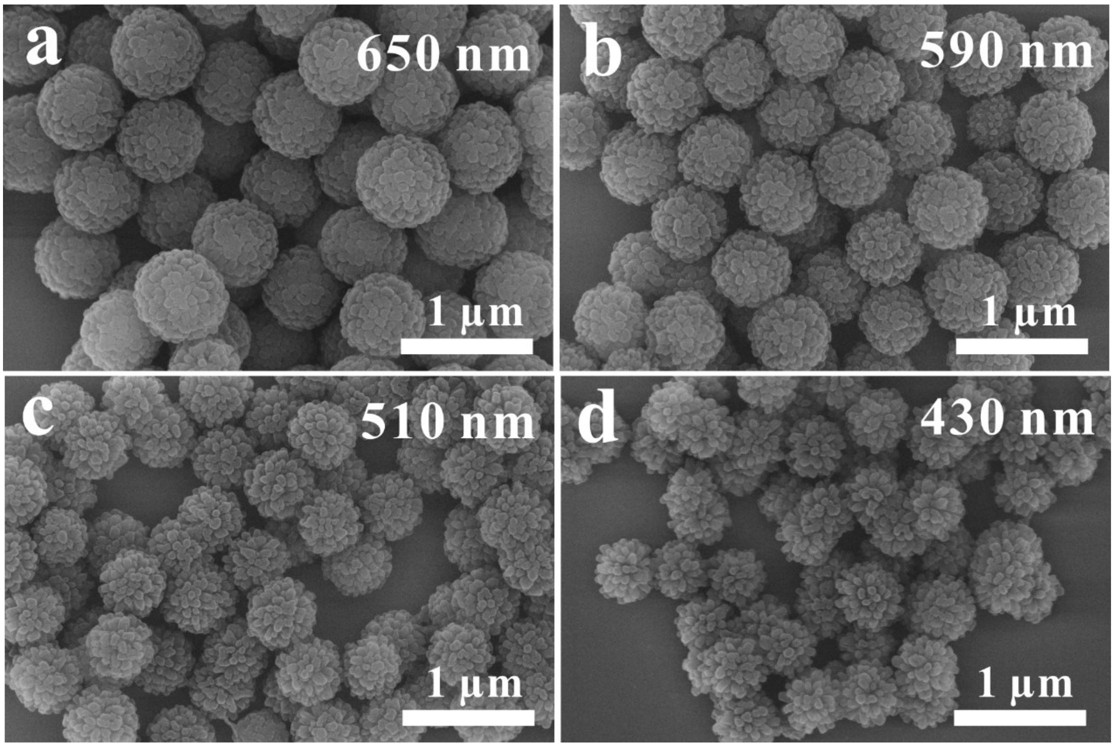


Figure S3. SEM images of the uniform HFH-COF-1 prepared with 1.9, 2.4, 2.9, and 3.4 M HAc, and the corresponding sizes: a) 650 nm, b) 590 nm, c) 510 nm, d) 430 nm.

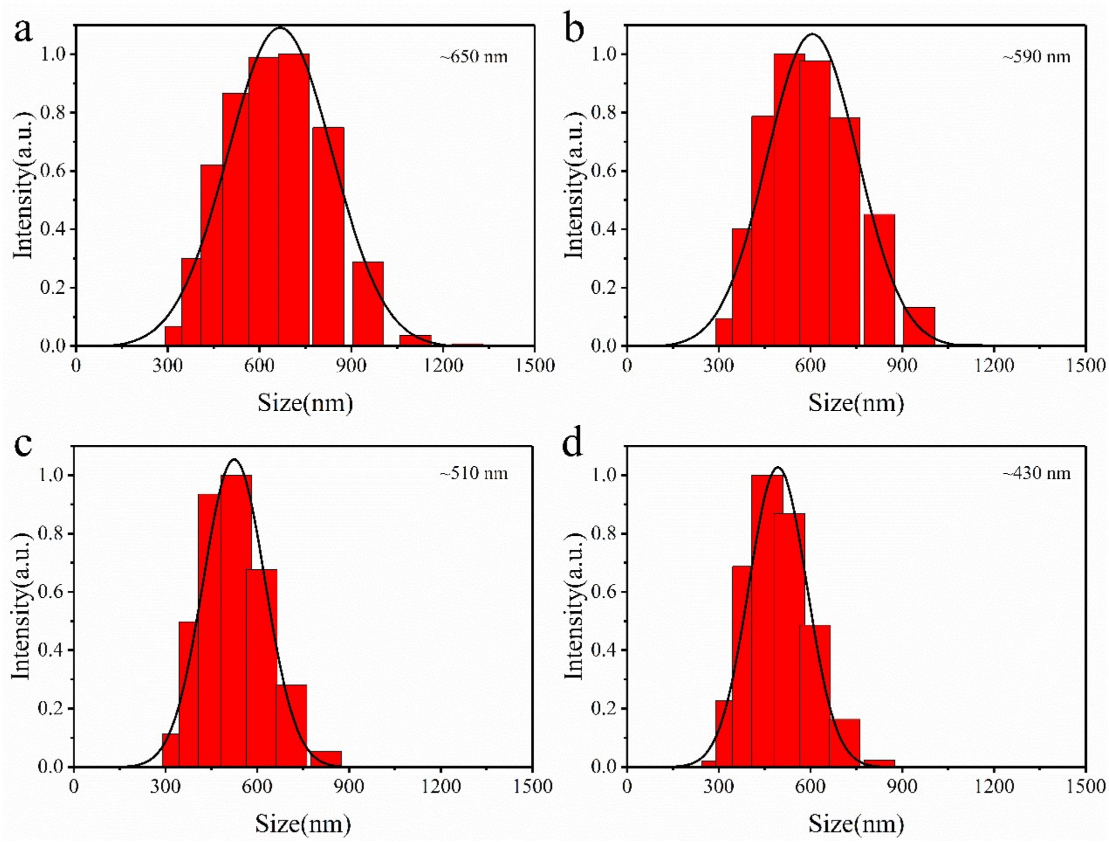


Figure S4. The DLS analysis of HFH-COFs-1 with different sizes (a: size of 650 nm, b: size of 590 nm, c: size of 510 nm, d: size of 430 nm).

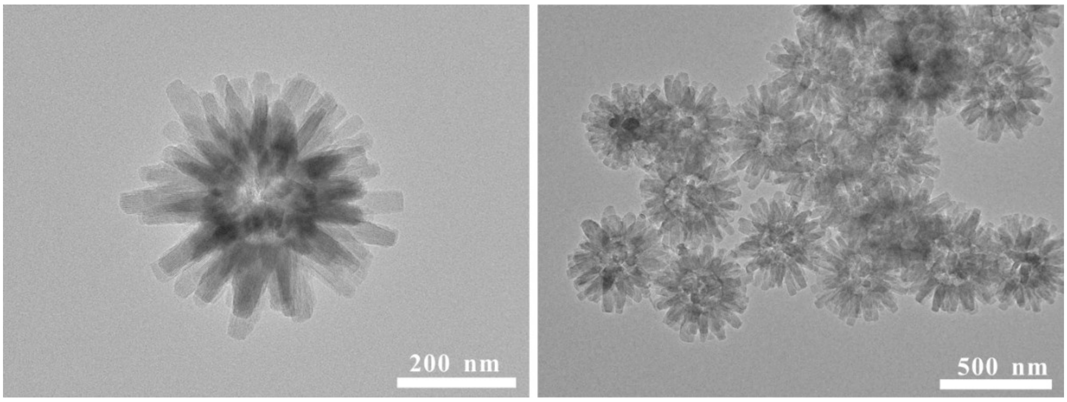


Figure S5. The TEM images of HFH-COFs-1 prepared with 3.9 M HAc.

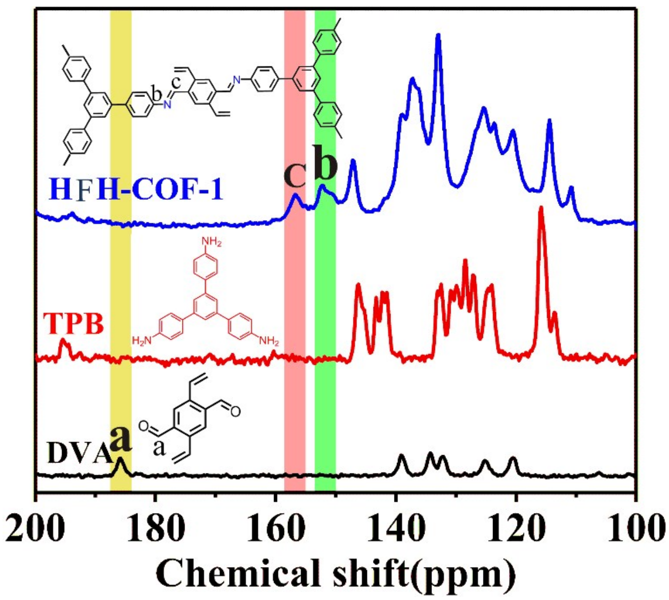


Figure S6. Comparison of solid-state 13C NMR spectra of HFH-COF-1 with the monomers.

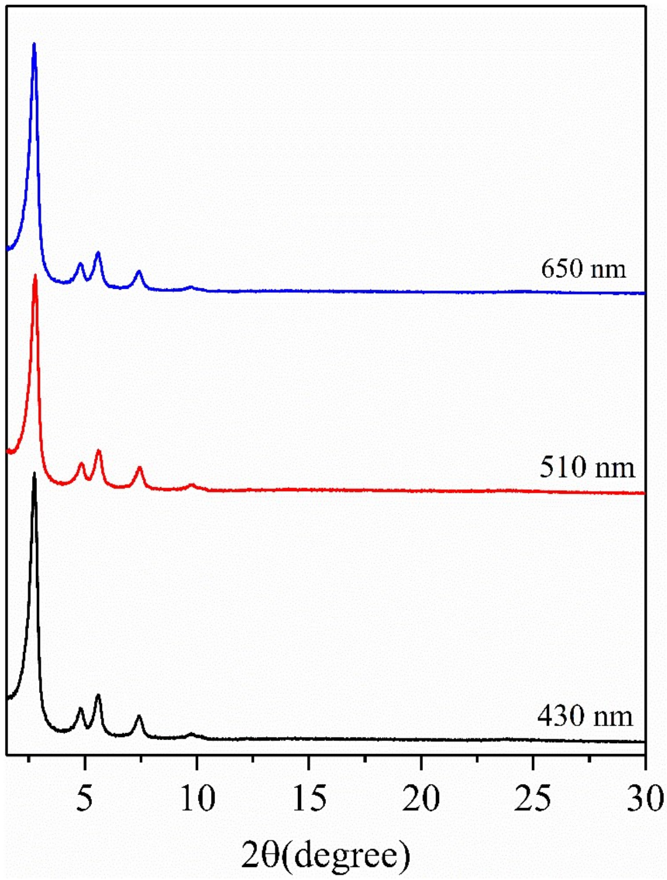


Figure S7. PXRD analysis of HFH-COFs-1 with different sizes (650 nm, 510 nm, and 430 nm).

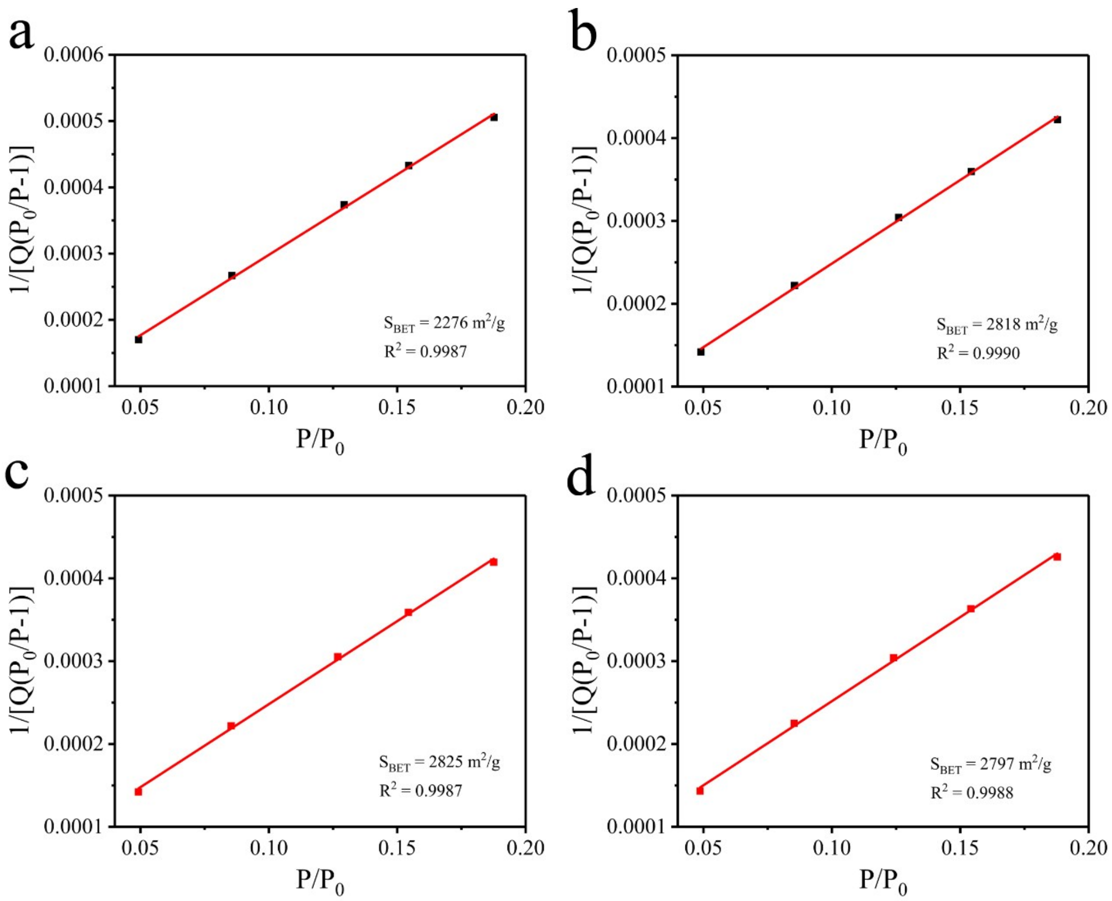


Figure S8. BET plot for the different-size HFH-COF-1 (a: size of 650 nm, b: size of 590 nm, c: size of 510 nm, d: size of 430 nm).

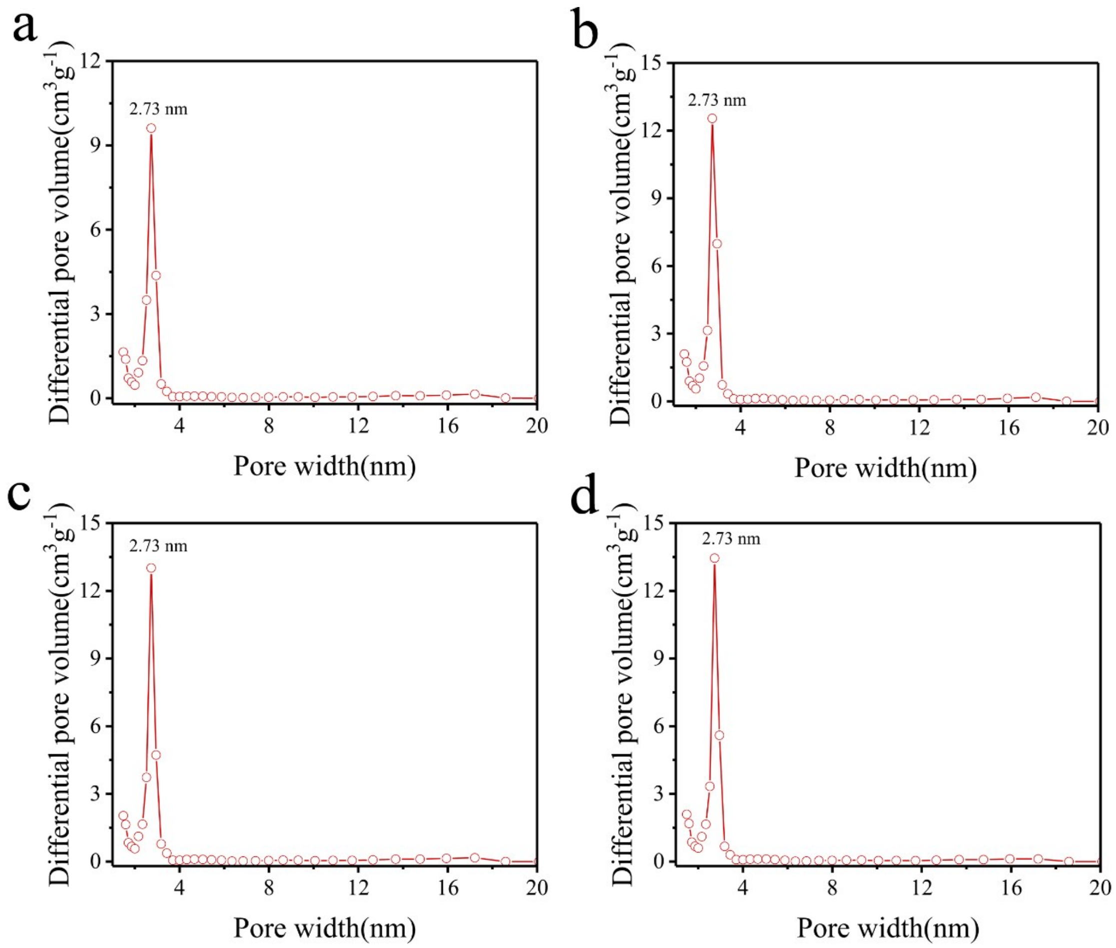


Figure S9. Pore size distribution profiles of HFH-COF-1 with different sizes (a: size of 650 nm, b: size of 590 nm, c: size of 510 nm, d: size of 430 nm). Pore size distributions of the HFH-COF-1 were calculated by using the NLDFT model.

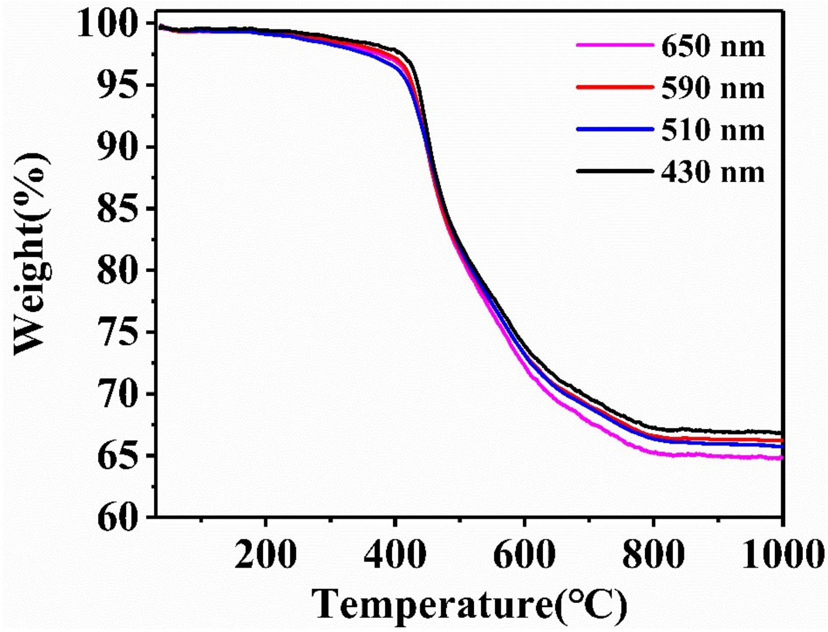


Figure S10. TGA curves of HFHCOF-1 with different sizes.

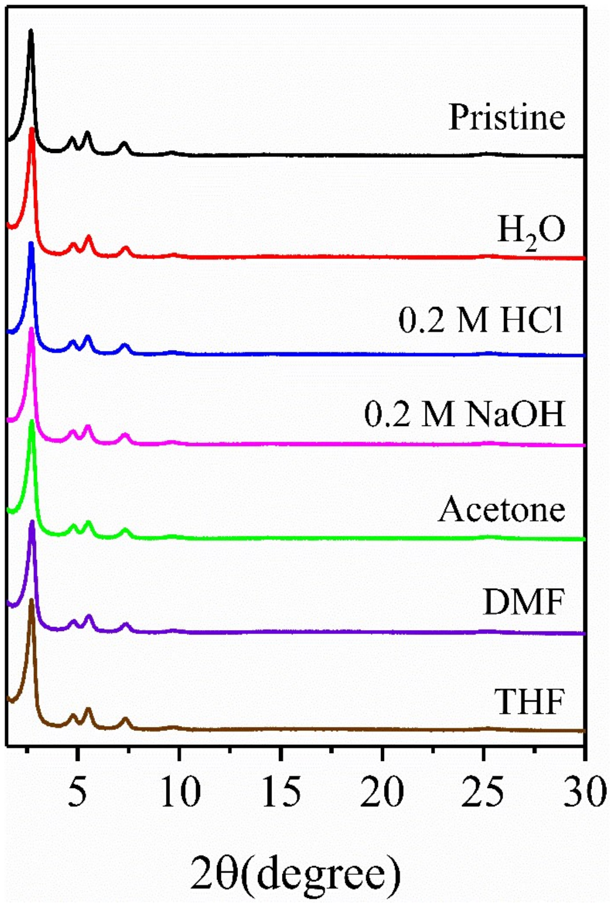


Figure S11. PXRD patterns of the HFH-COF-1 after treatment with different solvents for 48 h.

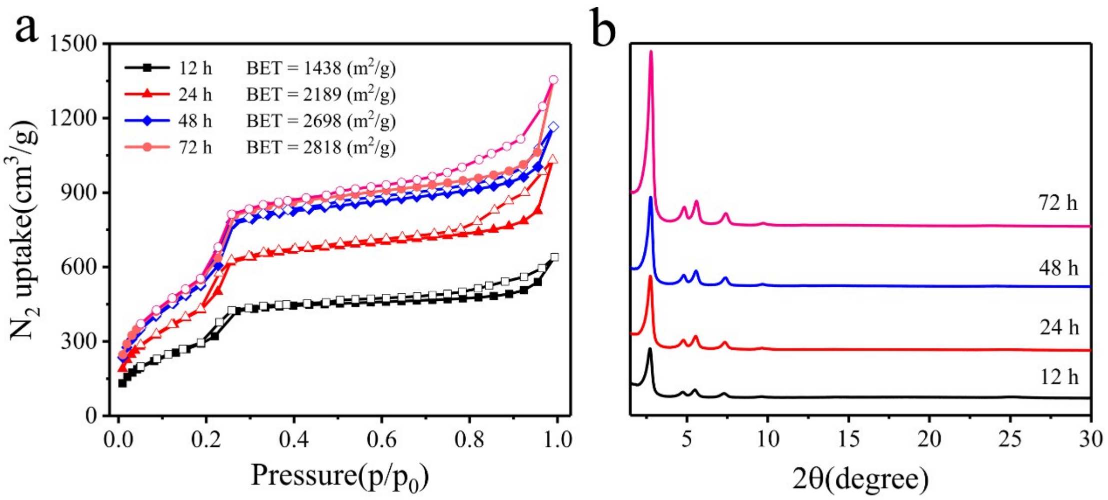


Figure S12. Time dependent BET and PXRD analysis of the formation of HFH-COF-1, reaction quenched after 12 h, 24 h, 48 h, and 72 h.

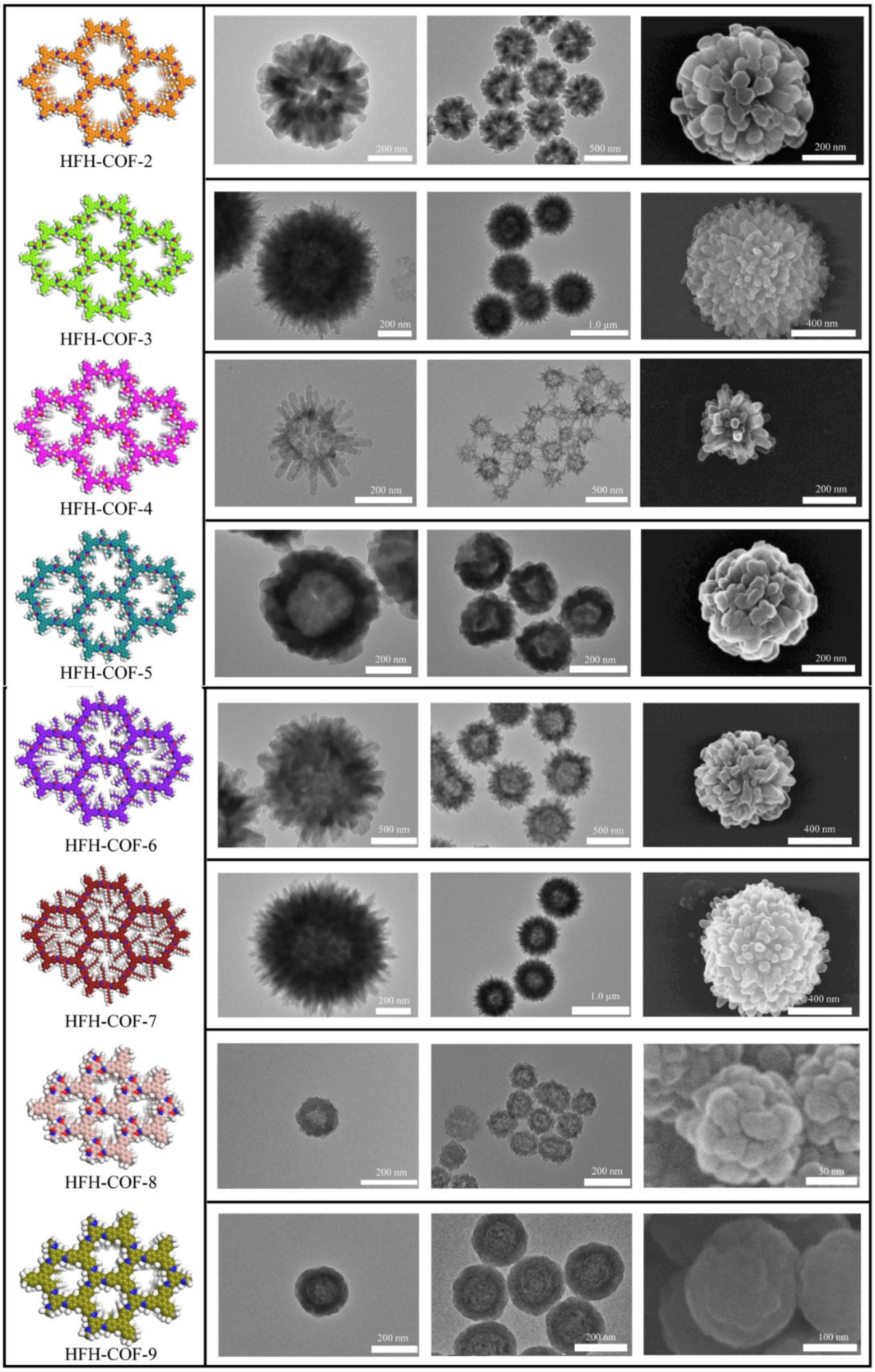


Figure S13. TEM and SEM images of a) HFH-COF-2, b) HFH-COF-3, c) HFH-COF-4, d) HFH-COF-5, e) HFH-COF-6, f) HFH-COF-7, g) HFH-COF-8, h) HFH-COF-9 synthesized in ACN solvent.

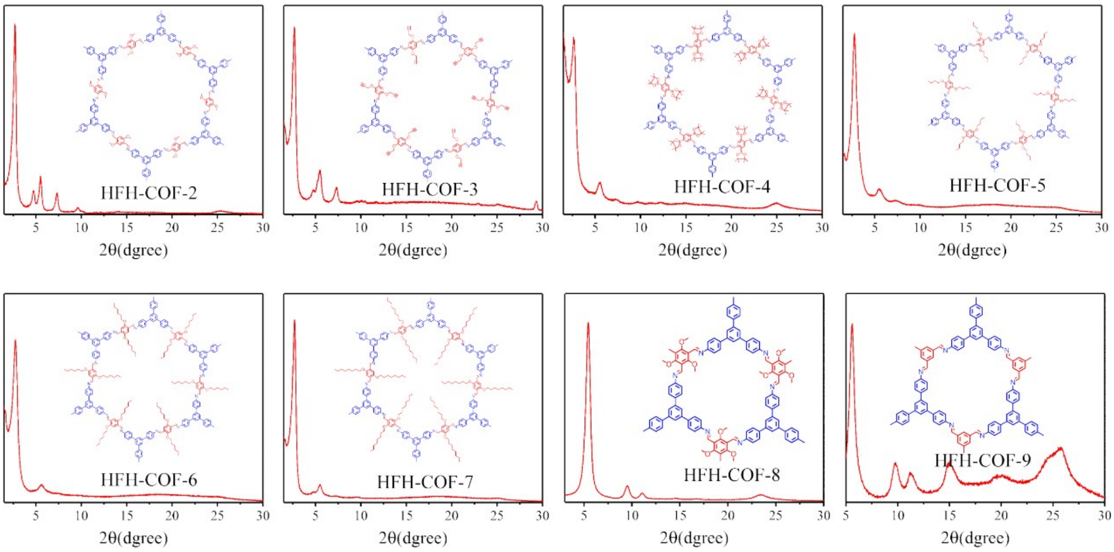


Figure S14. PXRD analysis of the different-class hierarchically flower-like hollow COFs.

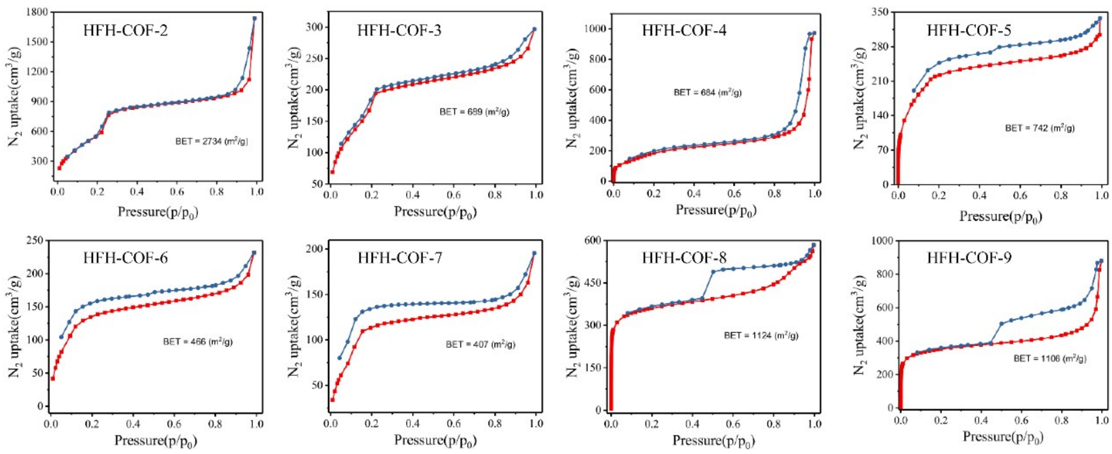


Figure S15. N_2 adsorption analysis of the different-class hierarchically flower-like hollow COFs.

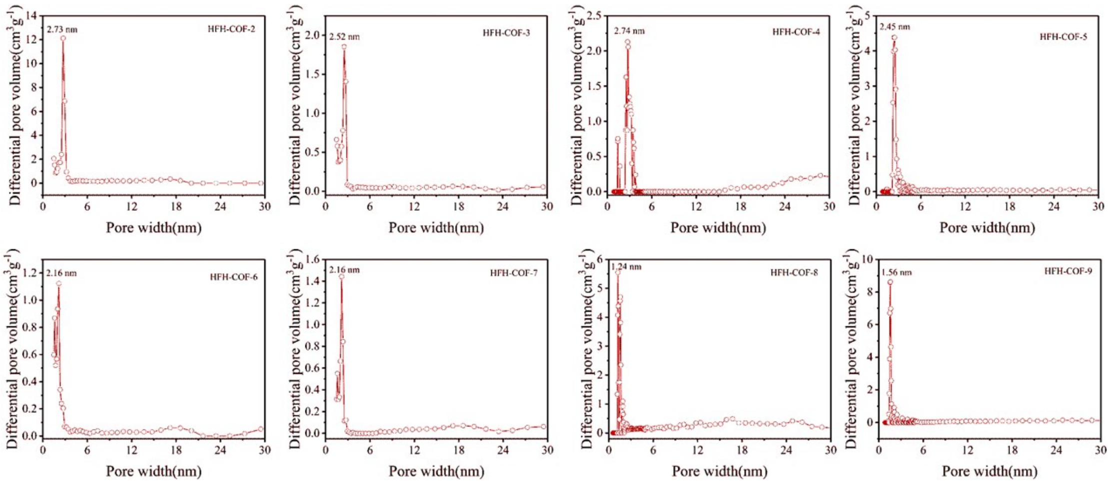


Figure S16. Pore size distribution profiles of the different-class hierarchically flower-like hollow COFs. Pore size distributions of the HFH-COF-1 were calculated by using the NLDFT model.

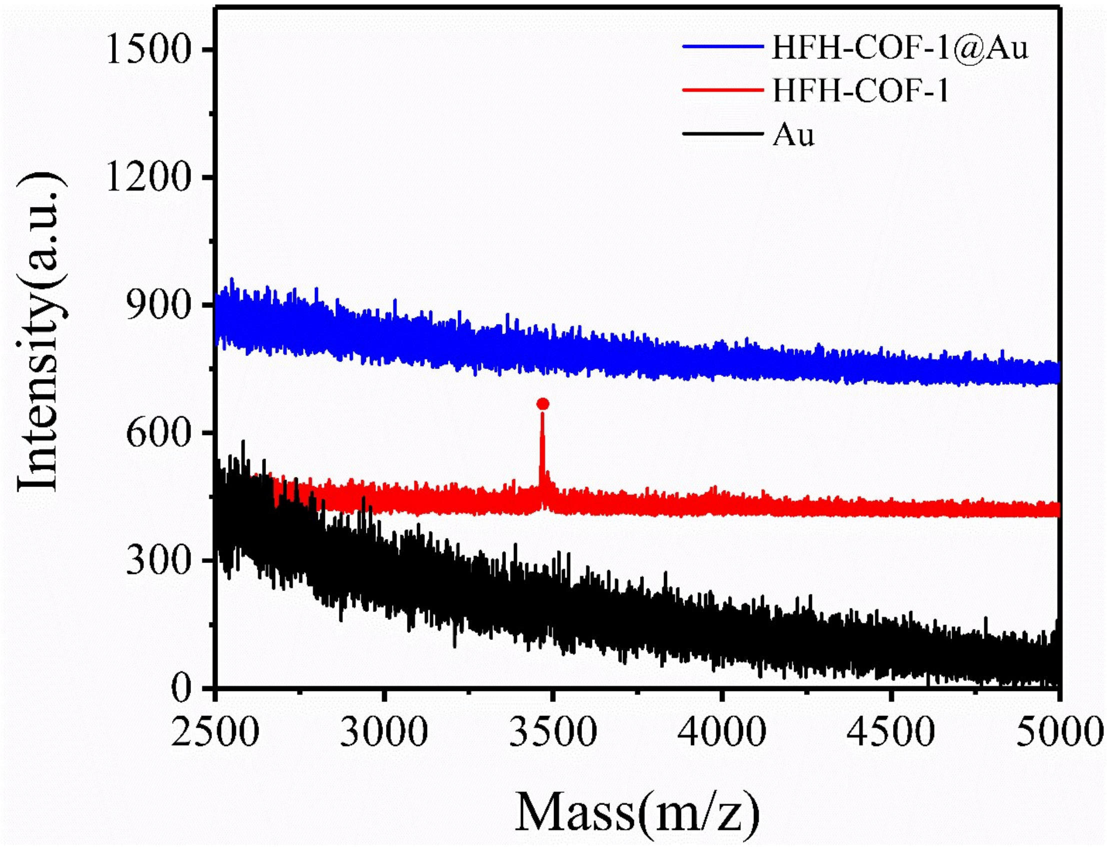


Figure S17. Mass spectra of the supernatant after enrichment of BNP (10 fmoL/μL, $[M+H]^+$, m/z = 3465.4) by HFH-COF-1@Au, HFH-COF-1, and Au, respectively (n=3, RSD < 10%). The peak of BNP is marked with red circles.

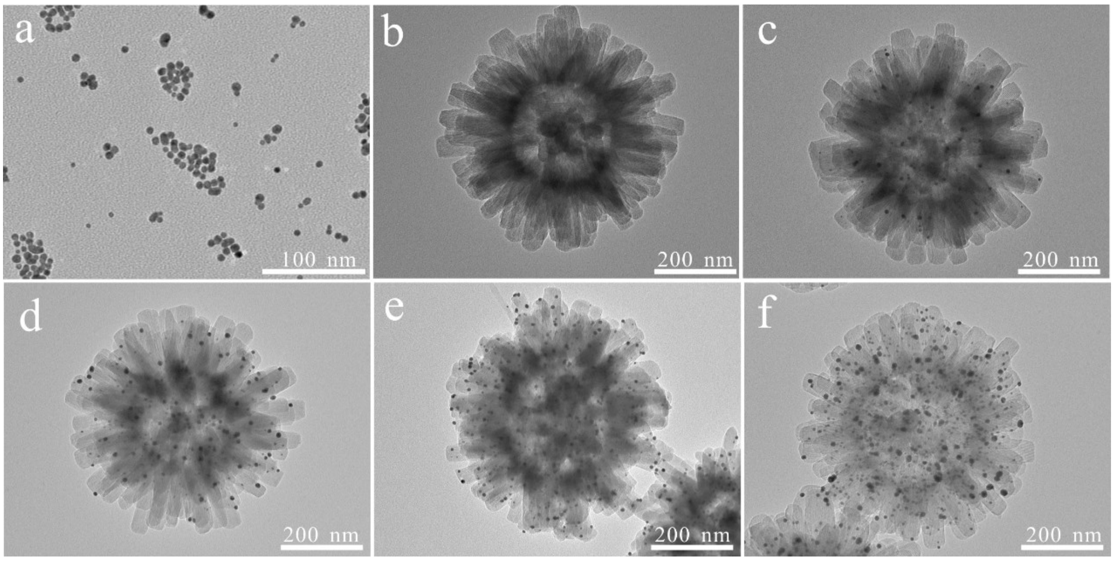


Figure S18. TEM images of a) Au nanoparticles, b) HFH-COF-1, c) HFH-COFs-1/Au (20:1.0, mg/mg), d) HFH-COFs-1/Au (20:1.5, mg/mg), e) HFH-COFs-1/Au (20:2.0, mg/mg), f) HFH-COFs-1/Au (20:2.5, mg/mg).

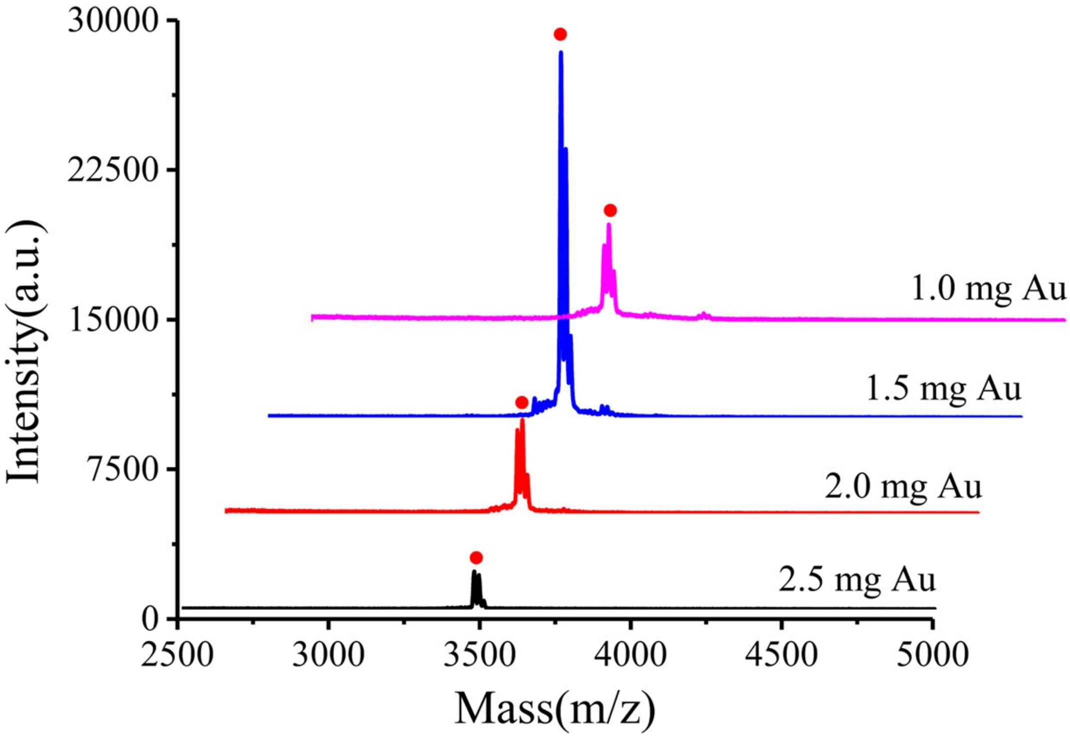


Figure S19. Mass spectra of BNP (10 fmoL/μL, $[M+H]^+$, m/z = 3465.4) after enrichment by HFH-COF-1@Au (HFH-COFs-1 loading different amounts of Au) (n=3, RSD < 10%). The peaks of BNP are marked with red circles.

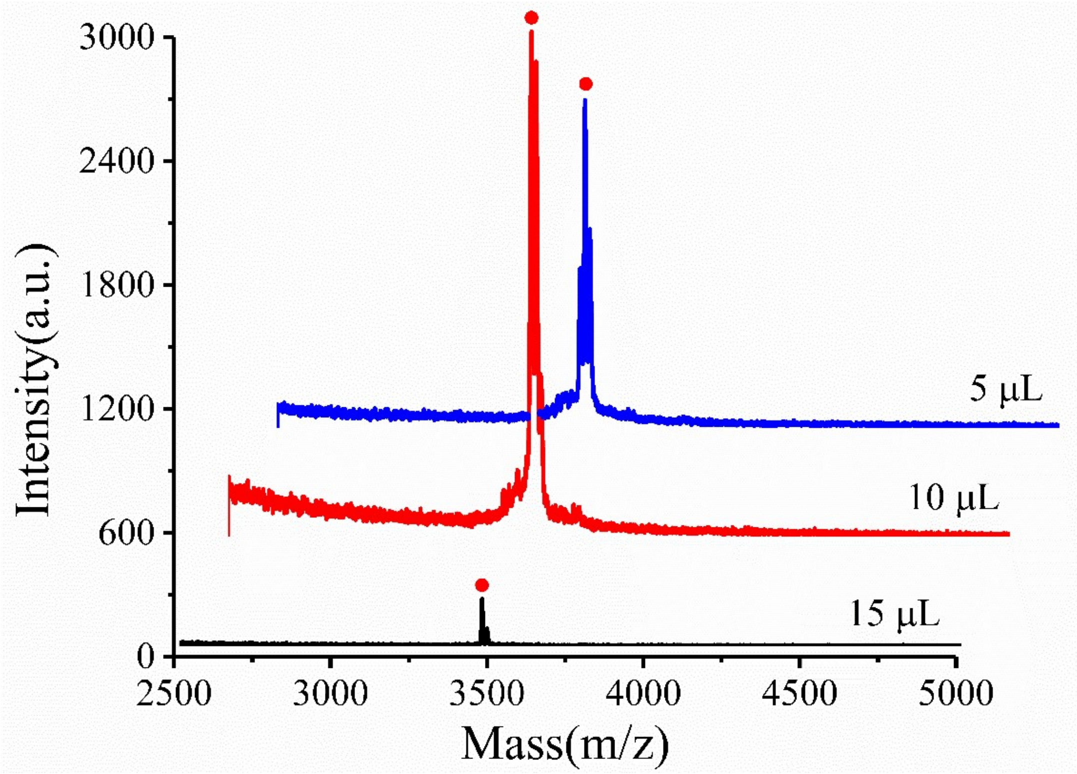


Figure S20. Mass spectra of the different volumes of DHB (30 mg/mL, 50%ACN:49%H_2O:1%H_3PO_4) eluted after enrichment of BNP (10 fmoL/μL, $[M+H]^+$, m/z = 3465.4) by HFH-COF-1@Au (HFH-COFs-1/Au (20:1.5, mg/mg)) (n=3, RSD < 10%). The peaks of BNP are marked with red circles.

Table S1. Atomistic coordinates for the AA-stacking mode of HFH-COF-1 optimized using Forcite method.

After refinement: space group P-1, a = b = 37.7898 Å, c = 3.7515 Å. Rwp= 8.52%, Rp= 7.10%			
Atom	x/a	y/b	z/c
H1	1.12618	-0.57656	-0.0106
H2	1.24325	-0.46759	0.09154
H3	1.13507	-0.45753	0.06549
H4	1.26688	-0.41208	0.42864
H5	1.31057	-0.33675	0.45786
H6	1.21944	-0.32128	-0.08881
H7	1.17695	-0.39593	-0.15588
H8	1.15837	-0.603	0.3146
H9	1.19128	-0.6454	0.29044
H10	1.29356	-0.54787	-0.26479
H11	1.26057	-0.50604	-0.26132
H12	1.25108	-0.26428	0.32358
H13	1.36426	-0.21657	0.22602
H14	1.31758	-0.11408	0.20414
H15	1.43067	-0.06664	0.08464
H16	1.3239	-0.5797	-0.000607
H17	1.29426	-0.7417	0.14549
H18	1.37439	-0.59488	-0.22488
H19	1.34478	-0.75651	-0.11197
H20	1.37527	-0.78933	-0.32792
H21	1.40983	-0.82971	-0.32879
H22	1.51347	-0.72953	0.20263
H23	1.47843	-0.68891	0.19633
H24	1.43126	-0.86483	0.06905
H25	1.5404	-0.87206	-0.06246
H26	1.54418	-0.75583	-0.19043
H27	1.40401	-0.92652	-0.22791
H28	1.3633	-1.00183	-0.15011
H29	1.46463	-1.00194	0.36495
H30	1.50515	-0.9266	0.31091
H31	1.59536	-0.83919	-0.43543
H32	1.67032	-0.80366	-0.46597
H33	1.67747	-0.69791	0.06365
H34	1.6038	-0.73371	0.12101
H35	1.74033	-0.68291	0.14796
H36	1.78695	-0.73489	-0.34865
H37	1.88625	-0.5918	0.02734

H38	1.93436	-0.64389	-0.44224
H39	1.24844	-0.68175	0.45171
H40	1.42092	-0.65556	-0.51179
H41	1.47509	-0.58612	-0.41264
H42	1.4424	-0.57594	-0.10624
H43	1.22599	-0.75971	0.01574
H44	1.19368	-0.7505	0.33518
H45	1.82967	-0.58699	0.33565
H46	1.76324	-0.62105	-0.23811
H47	1.77493	-0.57975	0.09564
H48	1.84376	-0.7393	-0.66158
H49	1.89783	-0.74747	-0.41813
H50	1.90935	-0.70664	-0.08021
H51	1.24765	-0.16983	0.39595
H52	1.23248	-0.23683	-0.14984
H53	1.19518	-0.22382	0.04676
H54	1.43421	-0.16083	0.03239
H55	1.48692	-0.10608	0.37456
H56	1.44962	-0.09292	0.56922
H57	1.07352	-0.49338	0.29194
H58	0.99931	-0.52718	0.23767
H59	0.99782	-0.62727	-0.34285
H60	1.07157	-0.59238	-0.32682
C1	1.12496	-0.51945	0.01861
C2	1.14428	-0.54336	0.01359
C3	1.18691	-0.52494	0.03911
C4	1.21047	-0.48177	0.0745
C5	1.19223	-0.4569	0.0887
C6	1.14942	-0.47624	0.05823
C7	1.20685	-0.5508	0.03009
C8	1.21793	-0.41089	0.1319
C9	1.25623	-0.39305	0.30452
C10	1.28112	-0.35004	0.32791
C11	1.26776	-0.32393	0.19507
C12	1.22943	-0.34117	0.02959
C13	1.20501	-0.38411	-0.00588
C14	1.18747	-0.59078	0.18009
C15	1.20625	-0.61491	0.17094
C16	1.24504	-0.59942	0.02196
C17	1.26451	-0.55974	-0.13123
C18	1.24551	-0.53578	-0.12865
C19	1.28271	-0.25332	0.25901
C20	1.31186	-0.20818	0.2316
C21	1.35396	-0.19419	0.21855

C22	1.38321	-0.15195	0.20479
C23	1.37006	-0.12239	0.19324
C24	1.32797	-0.13639	0.21188
C25	1.29873	-0.17859	0.22447
C26	1.39894	-0.07743	0.14204
C27	1.30194	-0.61251	0.00979
C28	1.31761	-0.64189	-0.00242
C29	1.29432	-0.68412	0.099
C30	1.31138	-0.7098	0.05772
C31	1.35102	-0.69456	-0.07523
C32	1.37442	-0.65234	-0.17429
C33	1.35728	-0.62672	-0.13559
C34	1.36674	-0.72379	-0.09317
C35	1.42413	-0.7363	-0.05605
C36	1.40491	-0.77633	-0.20336
C37	1.4248	-0.79944	-0.20492
C38	1.46426	-0.78298	-0.06074
C39	1.48348	-0.74275	0.0826
C40	1.46357	-0.71967	0.08226
C41	1.48533	-0.80761	-0.06155
C42	1.46337	-0.85034	0.00367
C43	1.48259	-0.87428	-0.00665
C44	1.52508	-0.85419	-0.06228
C45	1.54797	-0.81113	-0.11637
C46	1.52744	-0.78847	-0.12661
C47	1.45805	-0.92016	0.036
C48	1.59332	-0.78951	-0.15227
C49	1.41754	-0.94223	-0.09033
C50	1.39433	-0.98518	-0.05123
C51	1.41125	-1.00719	0.10334
C52	1.45161	-0.98563	0.22986
C53	1.47473	-0.94248	0.1983
C54	1.61292	-0.80888	-0.31572
C55	1.65561	-0.78865	-0.33631
C56	1.67958	-0.7489	-0.19571
C57	1.66016	-0.72898	-0.04012
C58	1.6176	-0.74926	-0.0151
C59	1.74992	-0.69684	-0.05435
C60	1.79424	-0.67859	-0.12134
C61	1.80867	-0.70343	-0.27004
C62	1.85058	-0.68916	-0.30409
C63	1.8791	-0.6482	-0.19718
C64	1.86458	-0.62325	-0.05214
C65	1.82272	-0.6375	-0.01819

C66	1.92368	-0.63026	-0.24657
C67	1.25359	-0.70131	0.26957
C68	1.41533	-0.63538	-0.3414
C69	1.44579	-0.59681	-0.2851
C70	1.22286	-0.7395	0.2045
C71	1.81098	-0.60788	0.12545
C72	1.78118	-0.60292	-0.01283
C73	1.86228	-0.71878	-0.4487
C74	1.89168	-0.72427	-0.30855
C75	1.25533	-0.18914	0.23484
C76	1.22605	-0.21855	0.03501
C77	1.42661	-0.14132	0.19152
C78	1.45601	-0.11147	0.38721
C79	1.07941	-0.5397	-0.01436
C80	1.05794	-0.52159	0.13796
C81	1.01539	-0.54102	0.11219
C82	0.99306	-0.57908	-0.06006
C83	1.01398	-0.59769	-0.21031
C84	1.05663	-0.57791	-0.1925
N1	1.29474	-0.28003	0.20758
N2	1.26308	-0.62532	0.02276
N3	1.38594	-0.05109	0.14453
N4	1.40538	-0.71101	-0.05184
N5	1.72324	-0.72937	-0.22875
N6	1.9493	-0.59856	-0.05765