Supplementary Information for

# Use of Confocal Fluorescence Microscopy to Compare Different Methods of Modifying Metal-Organic Framework (MOF) Crystals with Dyes

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## 1. Experimental

### *1.1 Materials*

DMF was purchased from Fisher Scientific. DEF was purchased from MERCK. All the other chemicals were purchased from Aldrich. All chemicals and solvents were used as received without further purification.

### *1.2 Preparations of IRMOF-1 micro crystals*

The typical synthetic procedure of IRMOF-1 micro crystals is as follows: $Zn(NO_3)_2 \cdot 6H_2O$ (1.795 g, 6 m mol), 1,4-benzenedicarboxylic acid ($H_2BDC$) (0.3325 g, 2 m mol), and 50 mL *N*,*N*-diethylformamide (**DEF**) were mixed at room temperature. The thus obtained mother solution was sealed and placed in the oven at 60℃ for 100 h. Then transferred the mother solution to another oven at 105℃ and heated for 3h. After that, the mother solution still kept clear. Cooled down the mother solution by flowing cool water and placed it in the oven at 60℃ for another period of time, which decides the size of products. The time we adopted here is 24h. Then the products were separated, well washed with chloroform three times (operated in air), and dried under vacuum. The dried products were stored in Ar atmosphere glove-box.

### *1.3 Coprecipitation synthesis of fluorescein-loaded IRMOF-1 micro crystals*

The typical preparation of dye-loaded IRMOF-1 micro crystals using coprecipitation method was performed as follows:

33.2 mg (0.1 m mol) Fluorescein dye was added into the standard mother solution of IRMOF-1 at room temperature to get a colored mixture. Then mixture was treated following the synthetic procedure of IRMOF-1 micro crystals (see 1.2). The whole process should avoid light. In our lab, we use Aluminum foil to enwrap the container and protect the products.

***1.4 Diffusion preparation of fluorescein-loaded IRMOF-1 micro crystals***

A fluorescein-DEF solution with a same dye concentration as that in coprecipitation synthesis (see 1.3) was prepared by dissolving 0.1 m mol fluorescein dye in 50 mL DEF at room temperature. Then the as-prepared IRMOF-1 micro crystals which yielded from 50 mL standard mother solution (see 1.2) were soaked in the fluorescein-DEF solution for 24h under sustaining shaking. The products were separated, well washed with chloroform three times, and dried under vacuum. The whole process should avoid light.

***1.5 Preparations of IRMOF-3 micro crystals***

The typical synthetic procedure of IRMOF-3 micro crystals is as follows: $Zn(NO_3)_2$•$4H_2O$ (1.44 g, 5.5 m mol), 2-Aminobenzen-1,4-benzenedicarboxylic acid ($NH_2$-$H_2BDC$) (0.37 g, 2 m mol), and 50 mL *N*,*N*-dimethylformamide (**DMF**) were mixed at room temperature. The thus obtained mother solution was sealed and placed in the oven at 60℃ for 72 h. Then dissolved Surfactant hexadecyltrimethylammonium bromide (CTAB, 3.64g, 10 m mol, CTAB: $NH_2$-$H_2BDC$=5:1) into mother solution while keeping 60℃. CTAB are used to slow down the formation of IRMOF-3 crystals. Transferred the mother solution to another oven at 105℃ and heated for 3h. After that, cooled down the mother solution by flowing cool water and placed it in the oven at 105℃ for 24h. Then the products were separated, well washed with chloroform three times (operated in air), and dried under vacuum. The dried products were stored in Ar atmosphere glove-box.

***1.6 Coprecipitation synthesis of fluorescein-loaded IRMOF-3 micro crystals***

The typical preparation of dye-loaded IRMOF-3 micro crystals using coprecipitation method was performed as follows:

33.2 mg (0.1 m mol) Fluorescein dye was added into the standard mother solution of IRMOF-3 at room temperature to get a colored mixture. Then mixture was treated following the synthetic procedure of IRMOF-3 micro crystals (see 1.5). The whole process should avoid light. In our lab, we use Aluminum foil to enwrap the container and protect the products.

***1.7 Diffusion preparation of fluorescein-loaded IRMOF-3 micro crystals***

A fluorescein-DMF solution with a same dye concentration as that in coprecipitation synthesis (see 1.6) was prepared by dissolving 33.2 mg (0.1 m mol) fluorescein dye in 50 mL DMF at room temperature. Then the as-prepared IRMOF-3 micro crystals which yielded from 50 mL standard mother solution (see 1.5) were soaked in the fluorescein-DMF solution for 24h under sustaining shaking. The products were separated, well washed with chloroform three times, and dried under vacuum. The whole process should avoid light.

***1.8 Chemical modification of dimethyl amino-terephthalate with FITC***

The typical modification procedure of dimethyl amino-terephthalate by FITC is as follows: 38.9 mg (0.1 m mol) FITC was dissolved into 2 mL DMF solvent to form a FITC-DMF solution. Added 33 μL (0.2 m mol) *N,N*-Diisopropylethylamine (DIPEA) to the FITC-DMF solution, shook for a little while. Then added 4.2 mg (0.02 m mol) dimethyl amino-terephthalate into the FITC-DMF solution, the as-gained solution was keeping at R.T. and under shaking for 24h. The products were gained by extracting DMF under vacuum at 100 $^{\circ}$C.

***1.9 Chemical modification of IRMOF-3 with FITC***

The typical chemical modification procedure of IRMOF-3 micro crystals by FITC is as follows:

8.0 mg as-prepared IRMOF-3 micro crystals were suspended in FITC-DMF solution [3.9 mg (0.01 m mol) FITC + 5 mL DMF + 3.3 μL (0.02 m mol) *N,N*-Diisopropylethylamine (DIPEA)]. DIPEA is used to speed the reaction between 2-amino group on the ligands and isothiocyanate group on FITC. Shook the suspension for 24 h, then separated the crystals and washed with fresh DMF for five times, immerged in DMF for 2 days and refreshed DMF every day. And then wash the crystals with chloroform for five times, immerged in chloroform over night, and dried under vacuum.

***2.0 Coprecipitation synthesis of FITC-loaded IRMOF-3 micro crystals***

The typical preparation of FITC-loaded IRMOF-3 micro crystals using coprecipitation method was performed as follows:

38.9 mg (0.1 m mol) FITC dye was added into the standard mother solution of IRMOF-3 at room temperature to get a colored mixture. Then mixture was treated following the synthetic

procedure of IRMOF-3 micro crystals (see 1.5) After 24 h heating at 105℃, no precipitation of crystals were observed. So prolong the heating at 105℃ for another 24h. Then the products were separated, well washed with chloroform three times (operated in air), and dried under vacuum. The dried products were stored in Ar atmosphere glove-box.

### *2.1 Characterization*

#### *2.1.1 Powder X-Ray diffraction (PXRD)*

All PXRD data were collected with the laboratory D8-Advance Bruker AXS diffractometer: CuKa radiation (λ = 1.54178); Göbel mirror; Θ–2Θ scan; 2Θ = 5 – 50°; step size = 0.0142 (2Θ); scan speed = 1 second/step; position sensitive detector; room temperature; α-$Al_2O_3$ was used as an external standard. The powder samples were filled into standard capillaries (0.7-mm diameter) and measured in Debye–Scherrer geometry. Background was automatically corrected using the standard Bruker data evaluation software (EVA 13.0.0.3, Bruker AXS 1996-2007). The phase purity was verified by the match search in the International Centre for Diffraction Data (ICDD, PDF-2 Release 2004) database and the samples were free of known crystalline impurities.

#### *2.1.2 Fluorescence Microscopy (FM)*

All FM micrographs were collected by microscope Olympus IX51. A cube with an excitation filter $\Lambda_{ex}$=450-480 nm and long pass Emission filter $\Lambda_{em}$=515—were chosen.

For the specimen preparing, a small amount of crystals were placed into a transparent plastic plate. 100 μL fresh ethanol was added to help the dispersing of crystals. Due to IRMOF-1 and IRMOF-3 are not stable in moisture, the products should not be put in air longer than hours.

#### *2.1.3 Confocal Laser Scanning Microscopy (CLSM)*

All CLSM micrographs were collected by True Confocal Scanner Leica TCS SP2. The specimen preparing method is same as that for Fluorescence Microscopy (see *2.1.2.*)

#### *2.1.4 Ultrahigh Vacuum Fourier Transform Infrared Spectroscopy (UHV-FTIRS)*

UHV-FTIRS experiments on pure and modified samples (IRMOF-1-mc, IRMOF-3-mc, and FITC-IRMOF-3-mc) were performed in an UHV apparatus, which combines a state-of-the-art vacuum IR spectrometer (Bruker, VERTEX 80v) with a novel UHV system (PREVAC). Powder samples were first pressed into a gold covered-stainless steel grid and then mounted on a sample holder, which was specially designed for the FTIR transmission measurements under UHV conditions. The base pressure in the measurement chamber amounted to 2 x $10^{-10}$ mbar. The

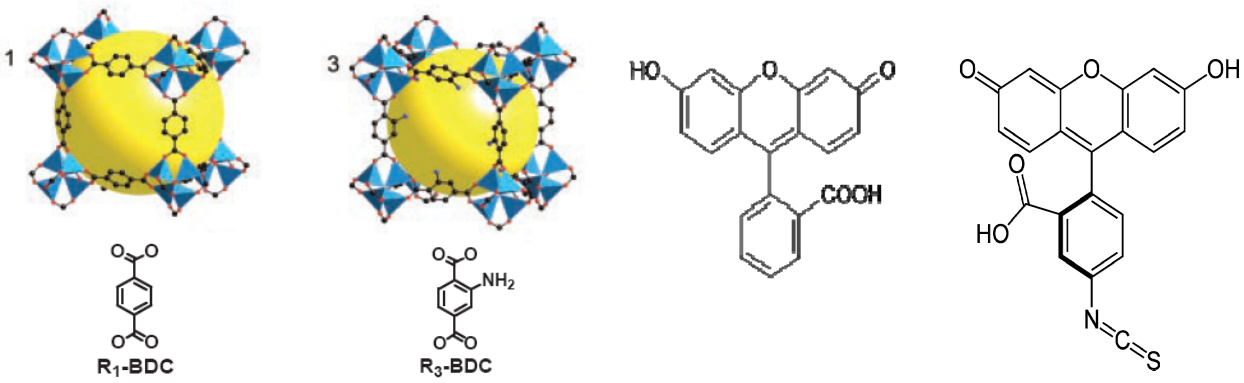
optical path inside the IR spectrometer and the space between the spectrometer and the UHV chamber were also evacuated to avoid atmospheric moisture adsorption, thus resulting in a high sensitivity and long-term stability. The samples were cleaned in the UHV chamber by heating to 600 K to remove all adsorbed species such as carbon-containing contaminants of solvent. All UHV-FTIR spectra were collected with 1024 scans at a resolution of 4 $cm^{-1}$ in transmission mode.

***2.1.5 Thermogravimetric Analysis (TGA)***

The analysis were performed using a TG/DTA EXSTAR 6200 SII thermogravimetric analyser from Seiko under 99.9999 % pure nitrogen (300 ml/min) gas stream, employing a heating rate of 5°C/min from room temperature to 600°C.

***2.1.5 1H-Nuclear Magnetic Resonance ($^1$H-NMR)***

$^1$H NMR spectra were recorded in deuterated solvents on Bruker DRX 250 spectrometers at 30°C. The chemical shifts, δ, are reported in ppm (parts per million).




**(a)** **(b)** **(c)** **(d)**

**Figure S1. Structures of (a) IRMOF-1, (b)IRMOF-3, (c) Fluorescein and (d) fluorescein-isothiocyanate (FITC). Note, the dye molecules are not planar!**

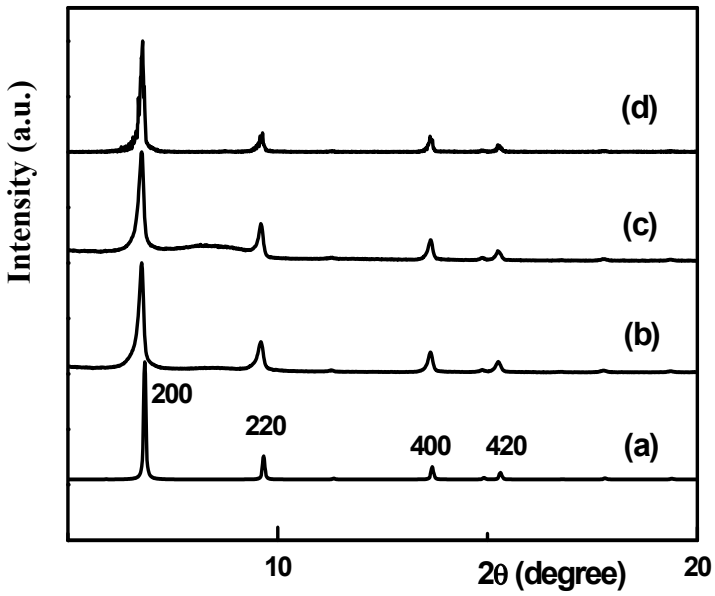



**Figure S2. PXRD patterns of (a) simulated IRMOF-1; (b) experimental empty IRMOF-1 micro crystals; (c) Fluorescein@IRMOF-1 prepared by coprecipitation method; (d) Fluorescein@IRMOF-1 prepared by diffusion method.**

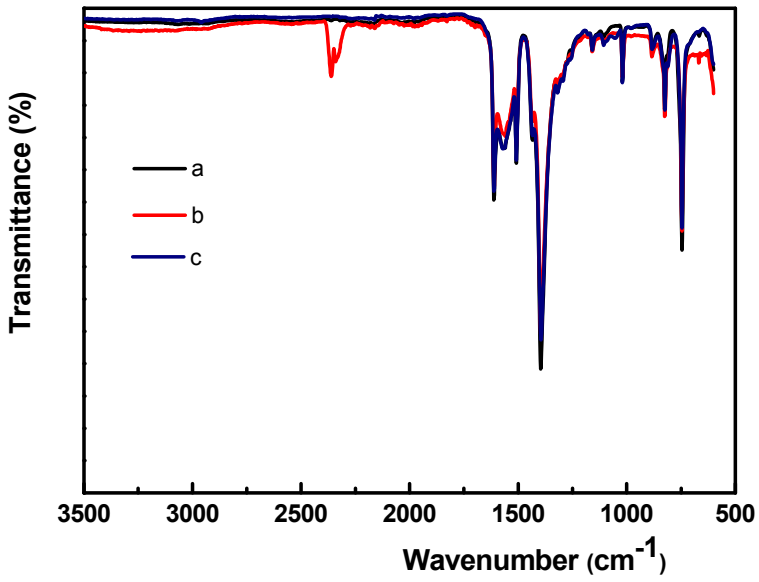



**Figure S3. Infrared Patterns of (a) Empty IRMOF-1-mc; (b) Fluorescein@IRMOF-1-mc prepared by coprecipitation method; (c) Fluorescein@IRMOF-1-mc prepared by diffusion method.**

In Figure S3, the shoulder at high wavenumbers and the peak above 2300 $cm^{-1}$ in the experimental spectrum are due to water and $CO_2$. Fluorescein used in our experiment has an

intensive band around 1595 $cm^{-1}$, and other broad bands around 847 $cm^{-1}$, 1113 $cm^{-1}$, 1481 $cm^{-1}$ especially. However, no obviously variety around these areas can be observed.

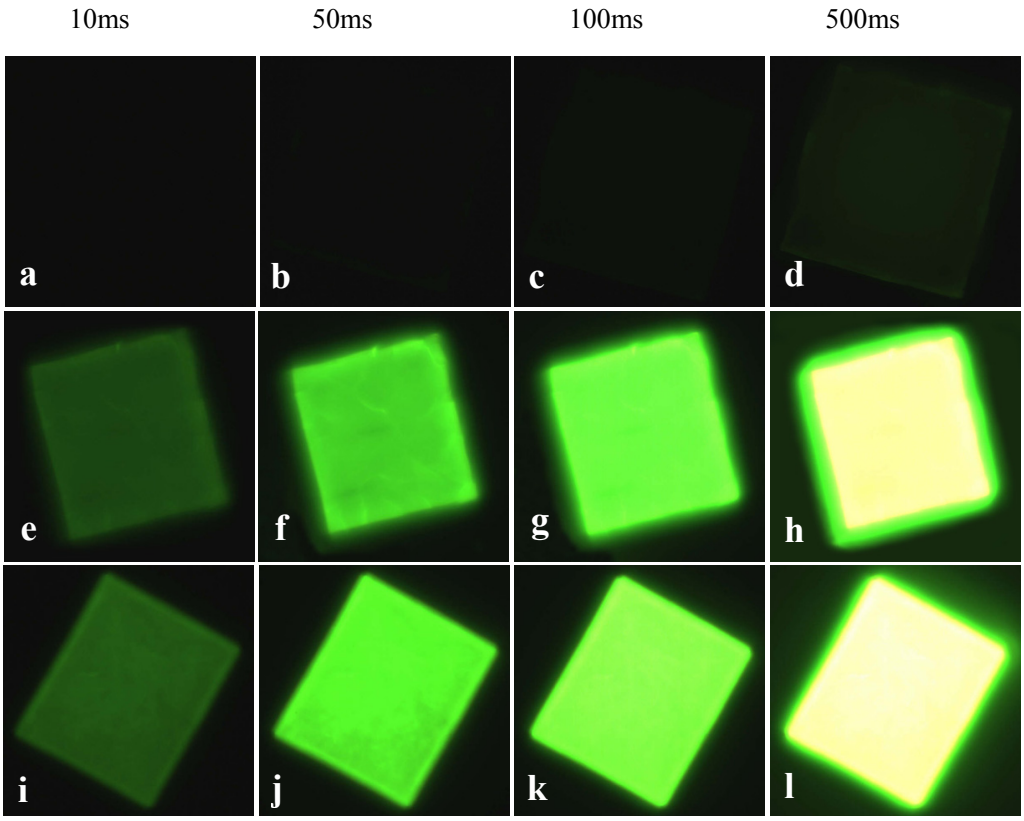



**Figure S4. Fluorescence Micrographs of empty IRMOF-1-mc (a-d), Fluorescein@IRMOF-1-mc prepared by coprecipitation method (e-h) and Fluorescein@IRMOF-1-mc prepared by the diffusion method (i-l): (a, e, i) ET=10 ms; (b, f, j) ET=50 ms; (c, g, k) ET=100ms; (d, h, l) ET=500 ms.**

IRMOF-1 micro crystals have auto-fluorescence which, however, is much weaker than that of fluorescein molecules. Herein, one can only observe a weak fluorescence of IRMOF-1 micro crystals when the exposure time (ET) is big enough. (>500ms, Figure S4-d) Exposure time, also called capture time, is the time of detector collecting emission fluorescence from specimen under Fluorescence Microscope. Bigger exposure time, stronger fluorescence intensity will be shown on the fluorescence graphs. In our communication, the fluorescence graphs of fluoreceice@IRMOF-1 were gained under an exposure time of 100 ms. Under that condition, no fluorescence of empty IRMOF-1 micro crystals can be observed (Figure S4-c).

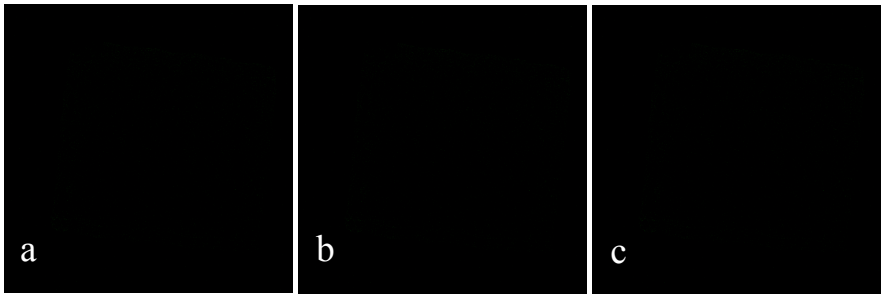



**Figure S5. Confocal laser scanning micrographs of empty IRMOF-1-mc. Three slices from top down each, detector (photomultiplier tube) voltage=600V. The slices chosen for a – c were in about 35 μm distance.**

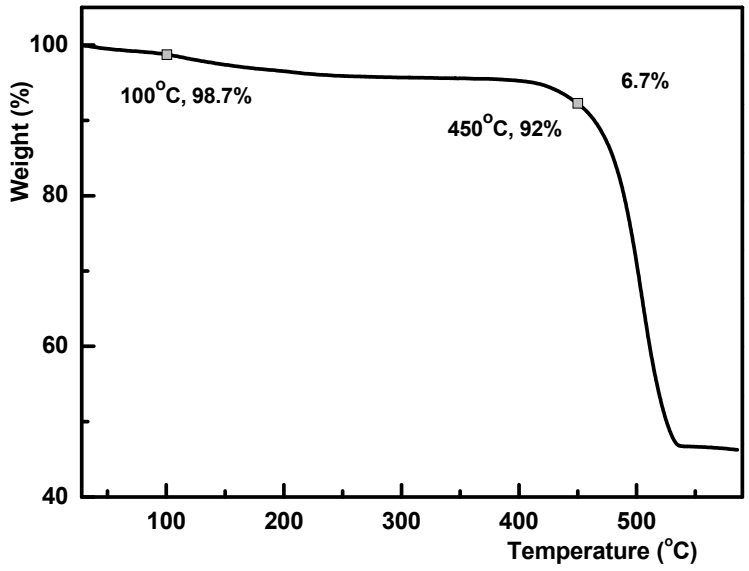



**Figure S6. TG curve of Fluorescein@IRMOF-1-mc prepared by the coprecipitation method.**

In Figure S6, the weight loss of ~6.7 % from 100 $^{o}$C to 450 $^{o}$C corresponds to the elimination of Fluorescein molecules in the crystal pores.

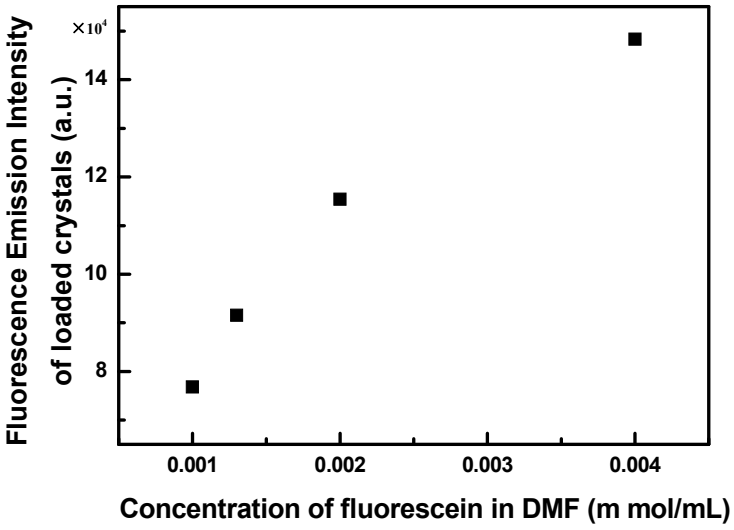



**Figure S7. The relationship between loadage of Fluorescein molecules inside IRMOF-1-mc and Fluorescein concentration in DMF solution.**

The Fluorescein@IRMOF-1-mc prepared by coprecipitation method from mother solutions with different Fluorescein concentrations were digested in $H_2O$ and then measured by Fluorescence spectroscopy. High fluorescence emission intensity illuminates high amount of fluorescein molecules loaded in the crystals. The typical concentration used in our communication is 0.002 m mol/mL.

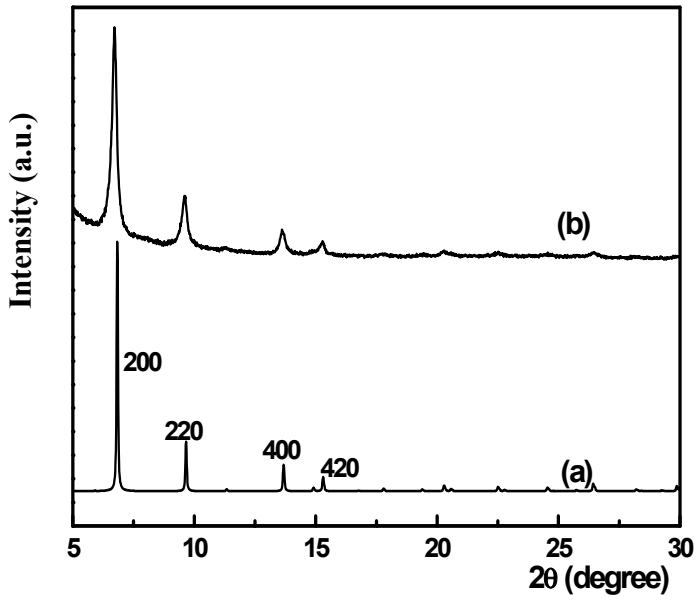



**Figure S8. PXRD patterns of (a) simulated IRMOF-1; (b) IRMOF-3 micro crystals.**

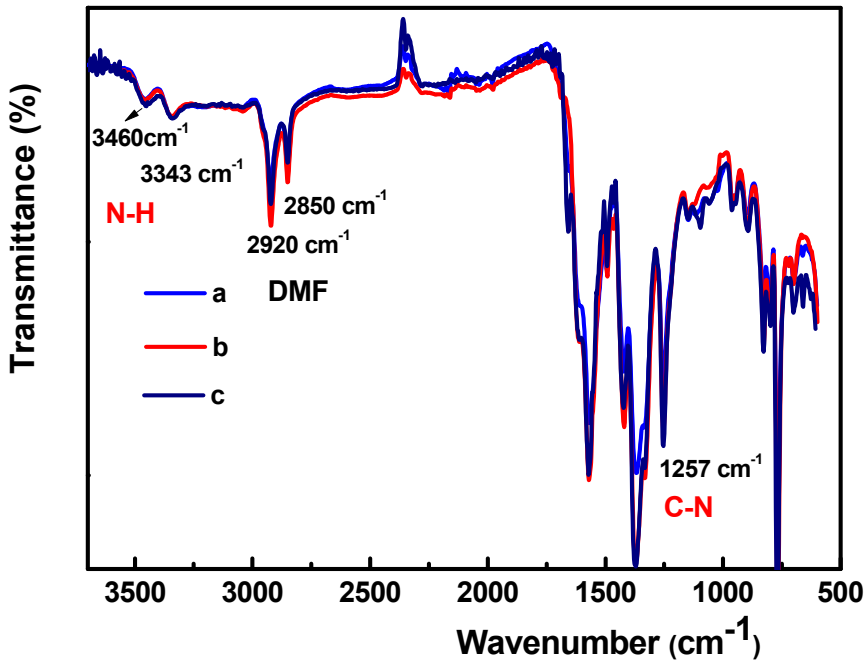



**Figure S9. Infrared Patterns of (a) Empty IRMOF-3-mc; (b) Fluorescein@IRMOF-3-mc prepared by coprecipitation method; (c) Fluorescein@IRMOF-3-mc prepared by diffusion method.** ν (N-H): 3340 $cm^{-1}$and 3360 $cm^{-1}$; ν (C-N): 1254 $cm^{-1}$;

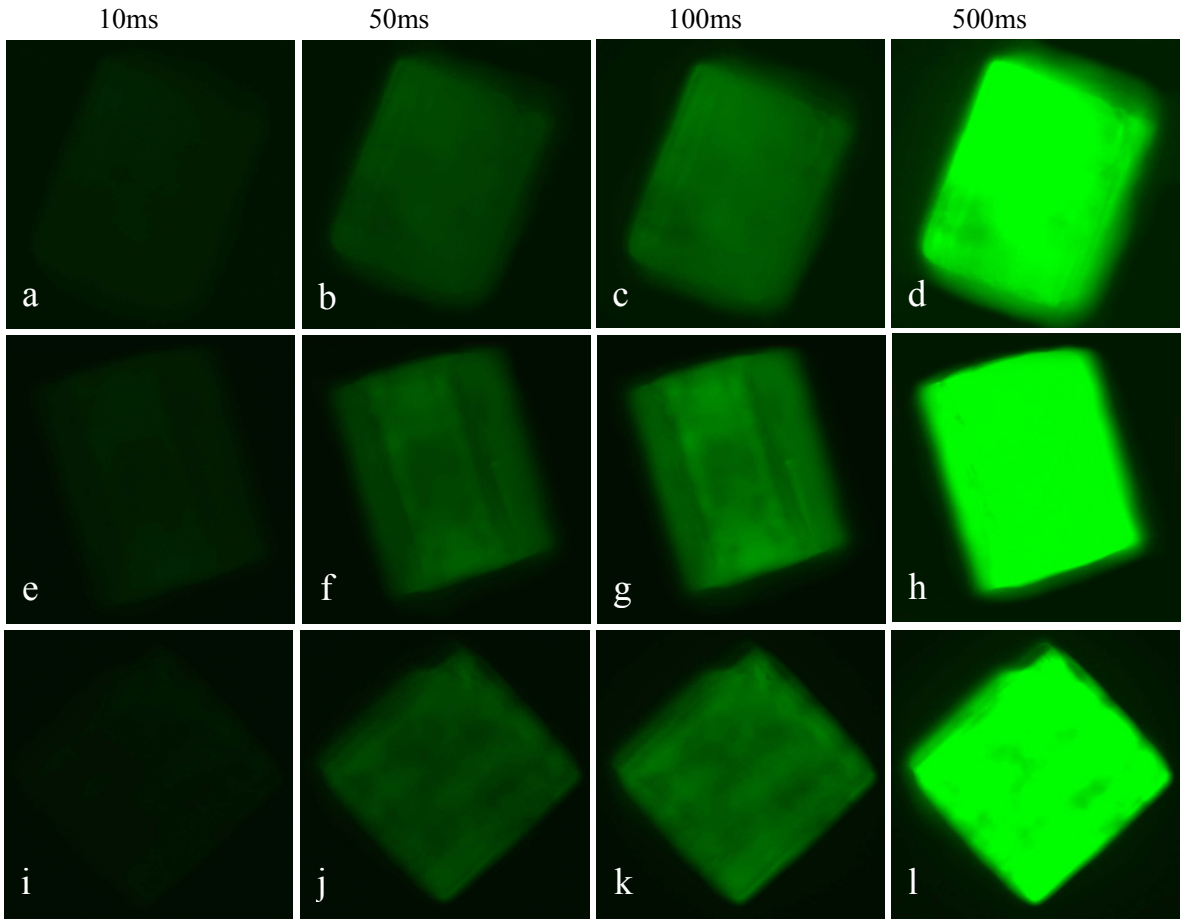


**Figure S10. Fluorescence Micrographs of empty IRMOF-3-mc (a-d), Fluorescein@IRMOF-3-mc prepared by coprecipitation method (e-h) and Fluorescein@IRMOF-3-mc prepared by the diffusion method (i-l): (a, e, i) ET=10 ms; (b, f, j) ET=50 ms; (c, g, k) ET=100ms; (d, h, l) ET=500 ms.**

As shown in Figure S10, IRMOF-3 crystals show stronger auto-fluorescence than IRMOF-1 crystals (Figure S10 a-d). No detectable increase in fluorescence was observed for IRMOF-3 samples after fluorescein modification.

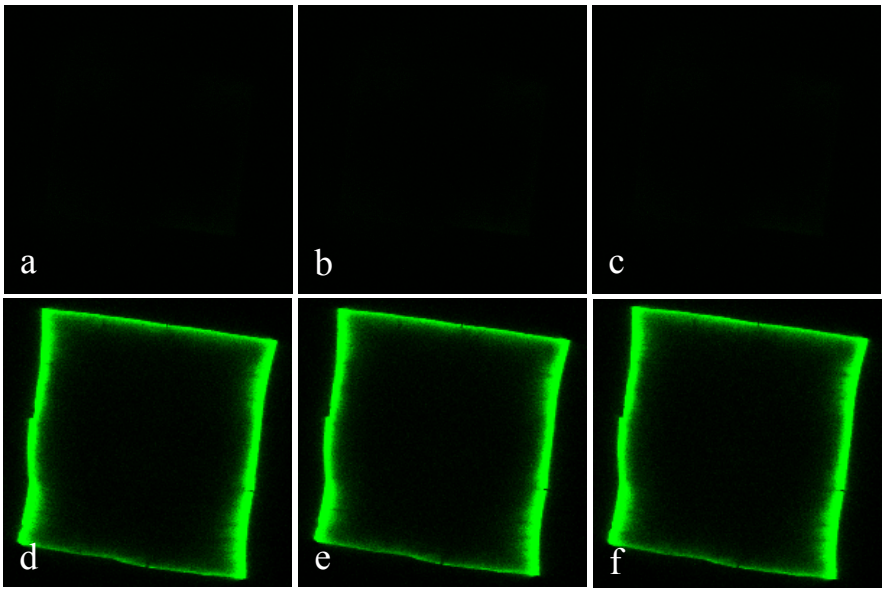
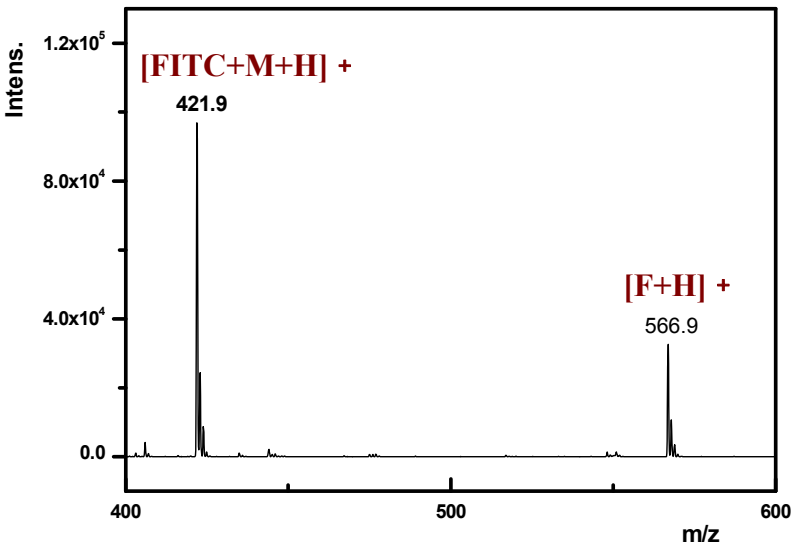


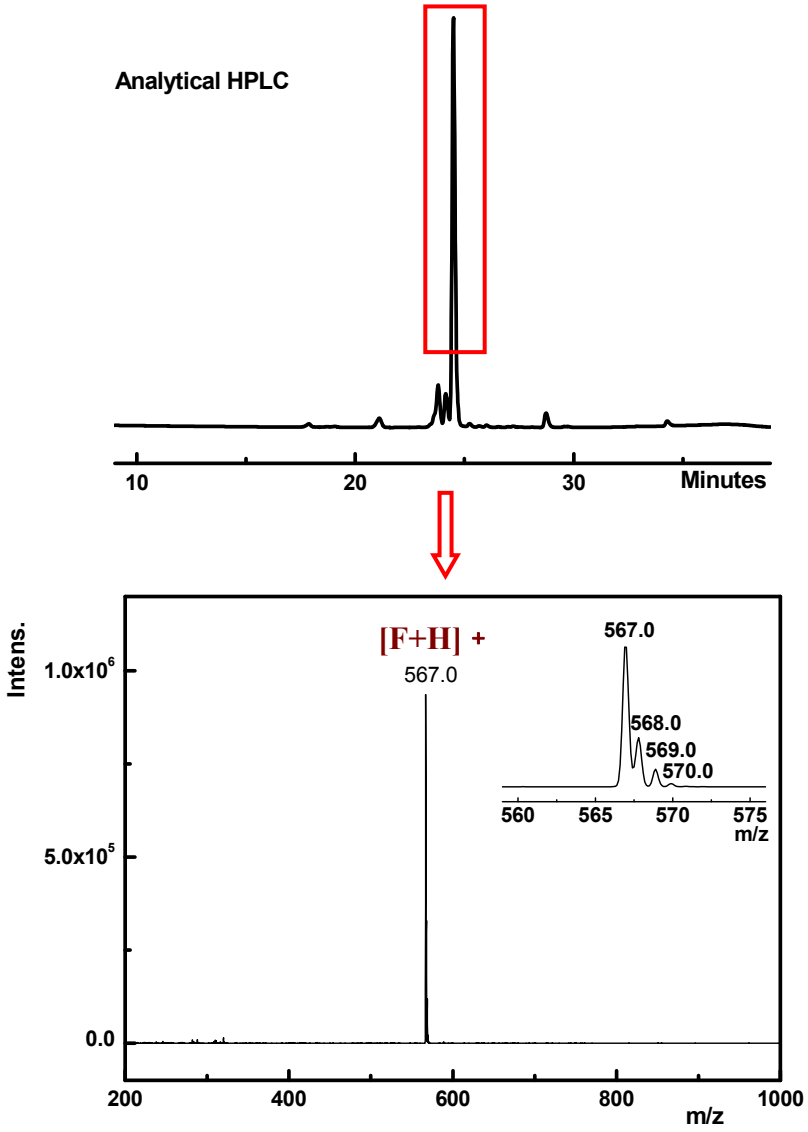

**Figure S11. Confocal laser scanning micrographs of empty IRMOF-3-mc (a-c) and Fluorescein@IRMOF-3-mc prepared by coprecipitation method (d-f).**

Three slices from top down each and the slices chosen for a – c and d – f were in about 35 μm distance**.** Detector (photomultiplier tube) voltage=600V.

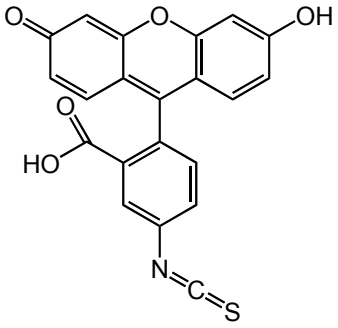
The IRMOF-3 crystal showed in Figure S11 d-f has visible cracks or defects, which fluorescein molecules can penetrate through, on the edges of the framework. More fluorescein molecules encapsulated in the frameworks comparing with the normal ones showed in our communication. However, this crystal gives a perfect example that fluorescein molecules will show very bright fluorescence comparing with empty IRMOF-3-mc under the condition used above (i.e. detector voltage=600V).


Intens.
1.2x10^5
8.0x10^4
4.0x10^4
0.0
[FITC+M+H] +
421.9
[F+H] +
566.9
400
500
600
m/z

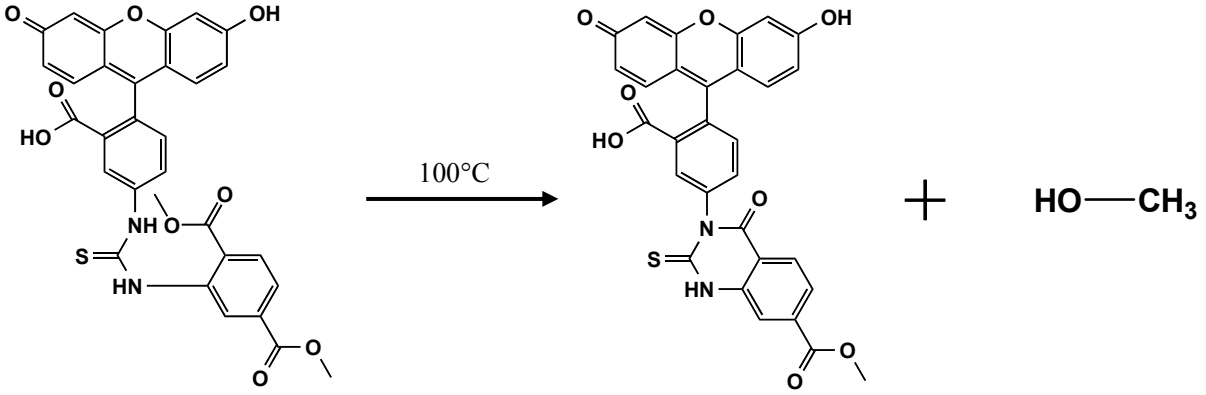
(a)


Analytical HPLC
10
20
30
Minutes
Intens.
1.0x10^6
5.0x10^5
0.0
[F+H] +
567.0
567.0
568.0
569.0
570.0
560
565
570
575
m/z
200
400
600
800
1000
m/z

(b)

**FITC =**  $[C_{21}H_{11}NO_5S]$ MW=389.38

**M = Methanol:** $[CH_4O]$ MW= 32.02621




**Fs**=$[C_{31}H_{22}N_2O_9S]$ MW=598.1046 **F** = $[C_{30}H_{18}N_2O_8S]$ MW=566.08

**Figure S12. (a) ESI-MS spectrum (positive mode) of FITC-NH-BDC-Me conjugates from a mixture of 1 equivalent dimethyl amino-terephthalate ($NH_2$-BDC-Me) and 5 equivalent FITC (see Experimental 1.8, 1.9), the products are dissolved in methanol. (b) Analytical HPLC of products from a mixture of 1 equivalent dimethyl amino-terephthalate ($NH_2$-BDC-Me) and 1 equivalent FITC, and ESI-MS spectrum (positive mode) of the main products. The products are dissolved in methanol.**

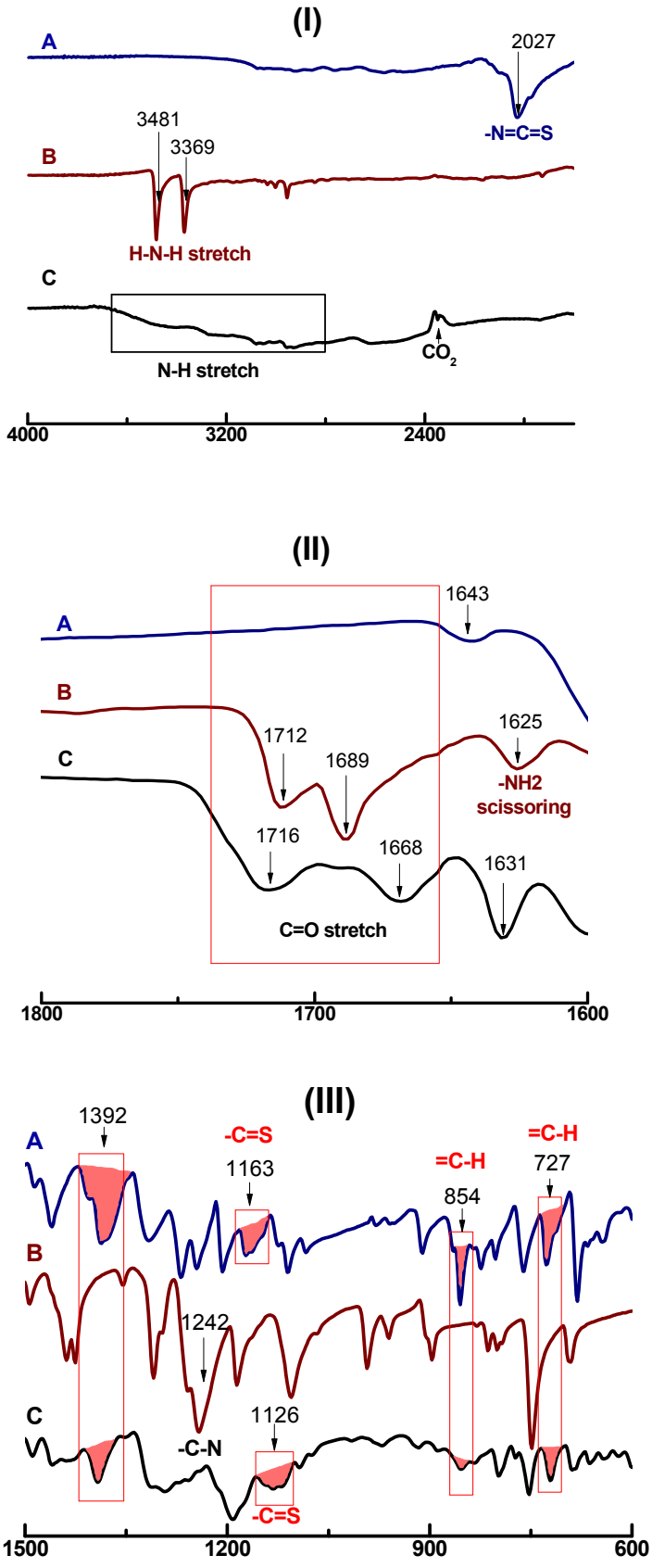



**Figure S13. Infrared Patterns of FITC (curve A), dimethyl amino-terephthalate ($NH_2$-BDC-Me, curve B) and FITC-NH-BDC-Me conjugates (curve C).**

Compared with pure FITC (curve A), the spectrum of FITC-NH-BDC-Me (curve C) presents a new broad IR band around 3000~3500 which assigned respectively to the ν(N–H) stretching vibrations of the secondary amino groups (-NH-) and two new bands at 1716, 1668 $cm^{-1}$ which are characteristic for the ν(-C=O) stretching of the ester groups and reveal the adding of $NH_2$-BDC-Me on to FITC. The disappearance of ν(-N=C=S) band at 2027 $cm^{-1}$ reveals the occurrence of chemical reactions on this group. (see Figure I, and II).

Compared with pure $NH_2$-BDC-Me (curve B), the spectrum of FITC-NH-BDC-Me (curve C) show new bands at 1392, 1163, 854 and 727 $cm^{-1}$ which are induced from the FITC molecules.

The shift of ν(C=S) band between FITC and FITC-NH-BDC-Me conjugate reveals the change on isothiocyanate group. (see Figure III)

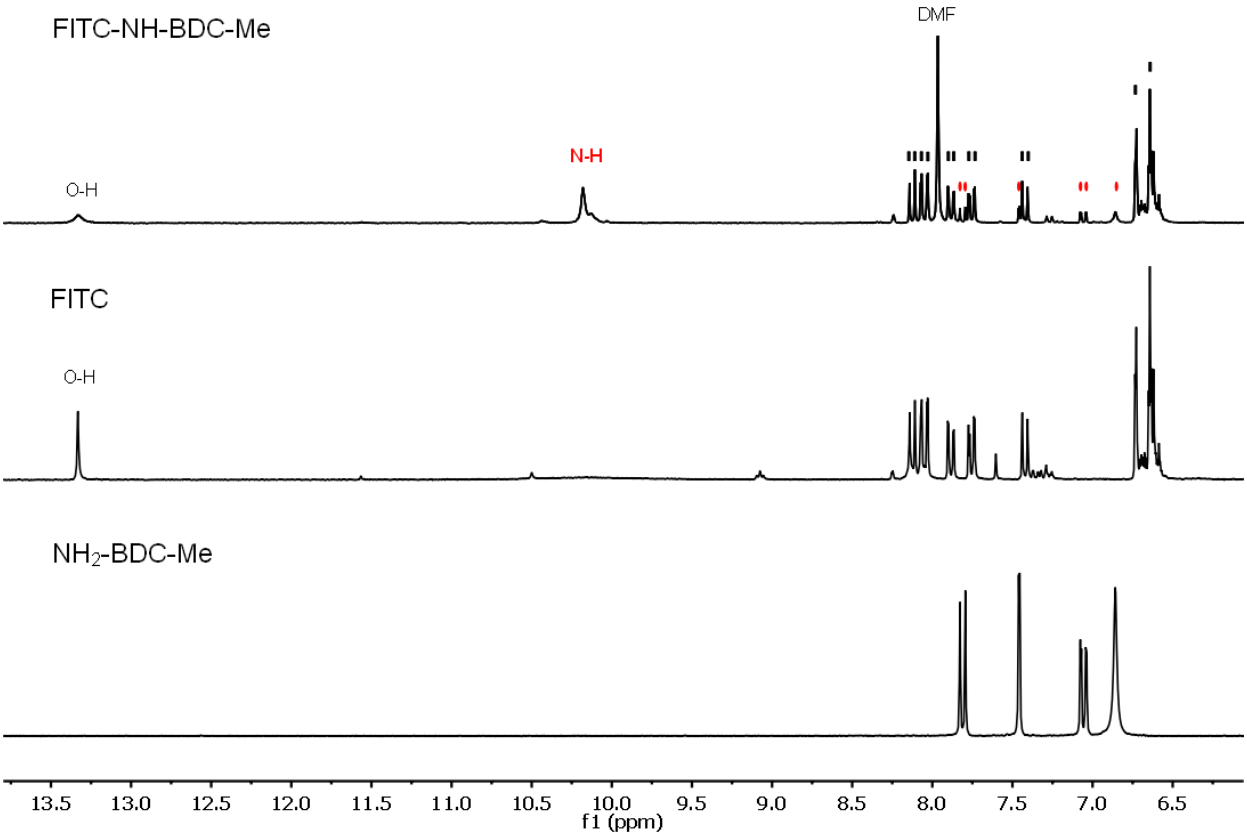
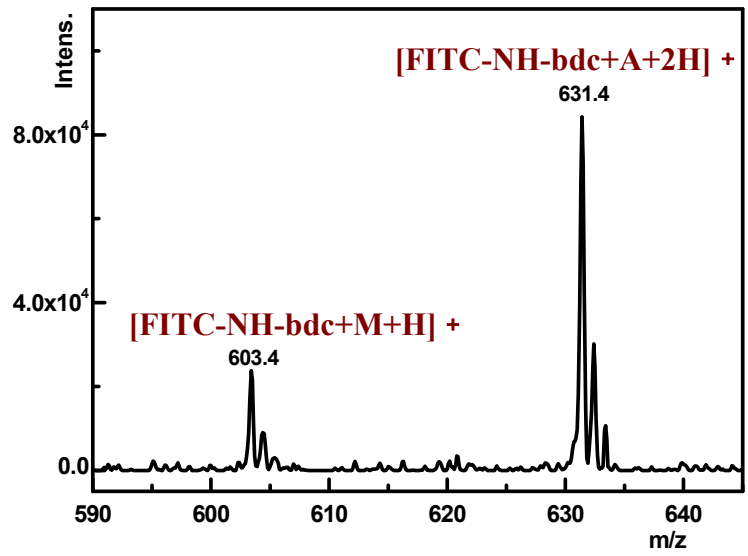
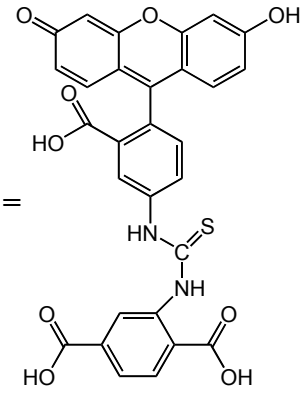



**Figure S14. $^1$H-NMR spectra of FITC-NH-BDC-Me conjugate, FITC and dimethyl amino-terephthalate ($NH_2$-BDC-Me). Black and red dots present signals of FITC and $NH_2$-BDC-Me, respectively.**




**FITC-NH-bdc =**  $[C_{29}H_{18}N_2O_9S]$, MW=570.0733

**M = Methanol:** $[CH_4O]$ MW= 32.02621

**A = Acetate Anion:** $CH_3COO^-$ MW=59.0133

**Figure S15. ESI-MS spectrum (positive mode) of FITC-IRMO-3 conjugates which were digested in a mixture of 0.1% acetic acid and methanol.**

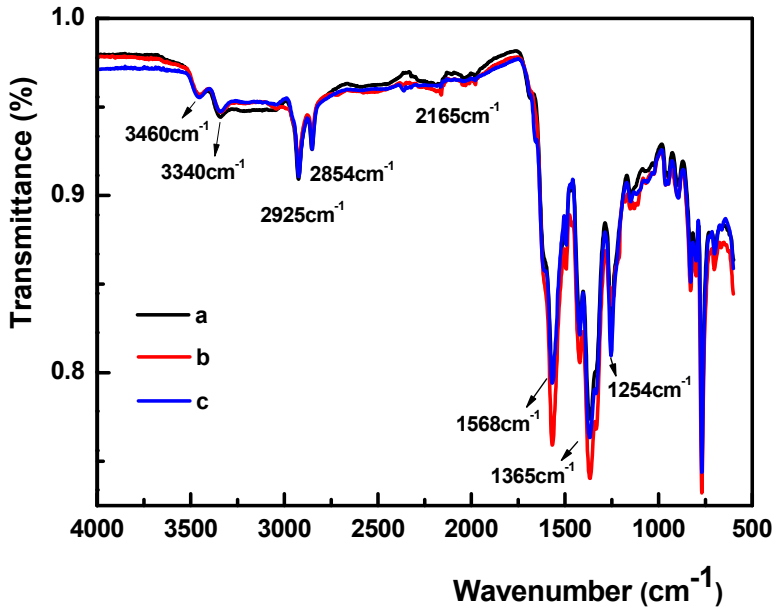



**Figure S16. Infrared Patterns of (a) empty IRMOF-3-mc, (b) FITC@IRMOF-3-mc prepared by coprecipitation method, (c) FITC-IRMOF-3-mc, chemical modified. Ar=benzene**

ν(N-H): 3340 $cm^{-1}$and 3360 $cm^{-1}$; ν(C-N): 1254 $cm^{-1}$; ν(DMF): 2854 $cm^{-1}$, 2925 $cm^{-1}$; ν(C-O): 1365 $cm^{-1}$; ν(C=C): 1568 $cm^{-1}$.

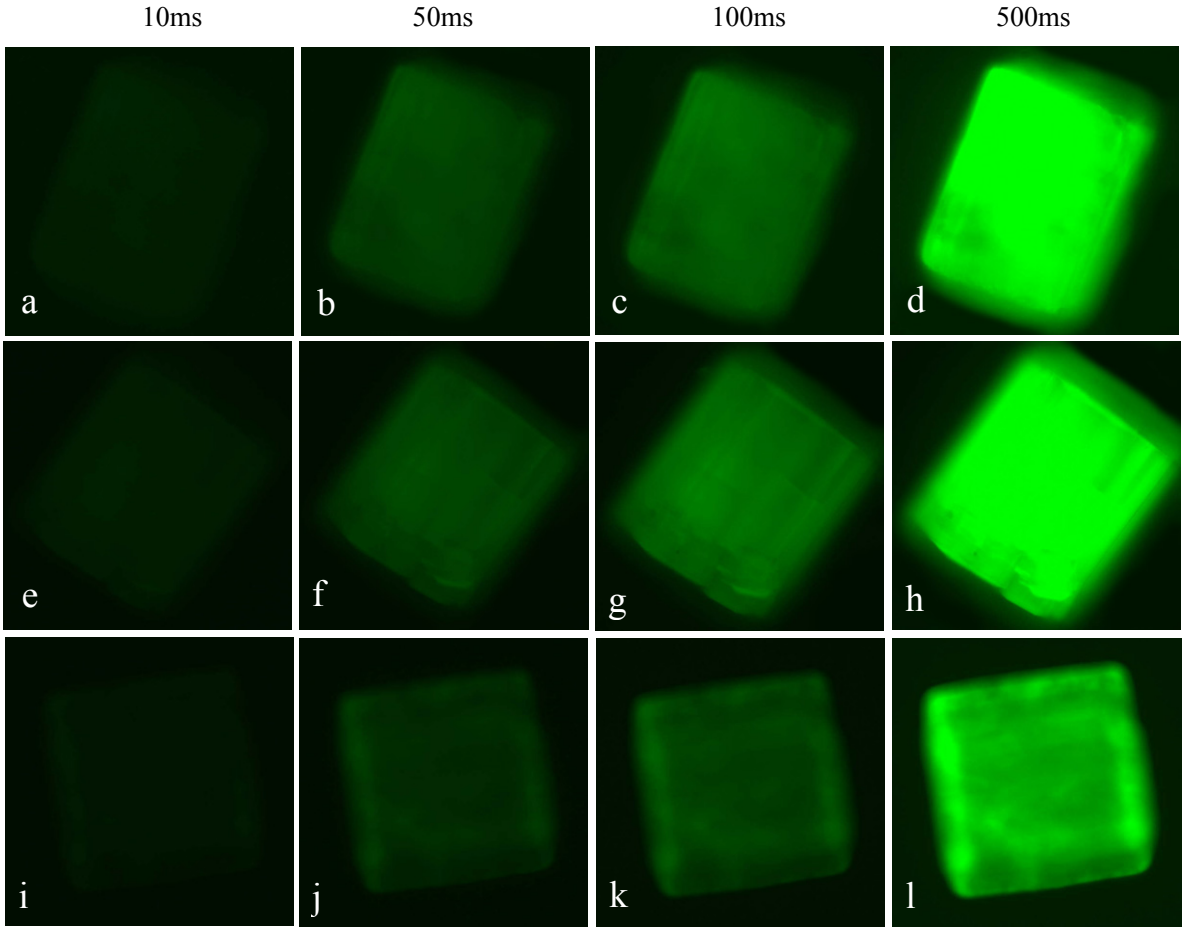


**Figure S17. Fluorescence Micrographs of empty IRMOF-3-mc (a-d), FITC@IRMOF-3-mc prepared by coprecipitation method (e-h) and FITC-IRMOF-3-mc prepared by the diffusion method (i-l): (a, e, i) ET=10 ms; (b, f, j) ET=50 ms; (c, g, k) ET=100ms; (d, h, l) ET=500 ms.**

As showed in Figure S17, IRMOF-3 crystals show stronger auto-fluorescence than IRMOF-1 crystals. No detectable increase in fluorescence was observed for IRMOF-3 samples upon FITC modification.

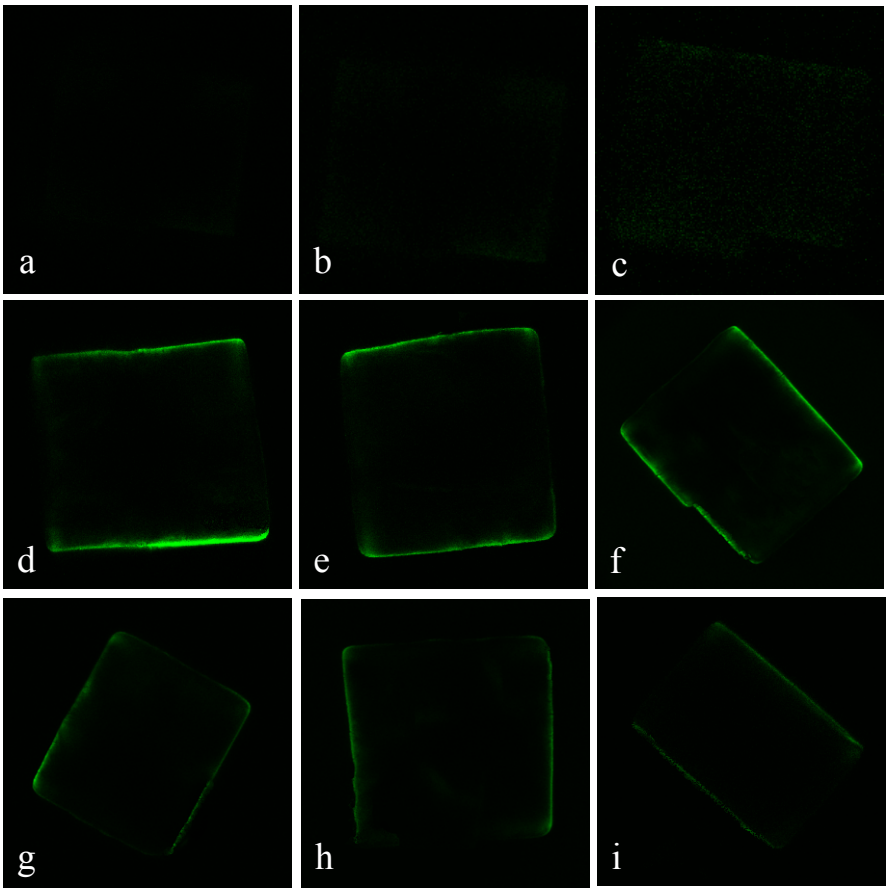



**Figure S18. Confocal laser scanning micrographs of empty IRMOF-3-mc (a-c): (a) Detector Voltage (DT) =600V, (b) DT=700V, (c) DT=800V, and different FITC-IRMOF-3 crystals (d-i): DT=600V.**

Under DT=600V, blank IRMOF-3-mc exhibits no fluorescence while FITC molecules exhibit strong fluorescence.

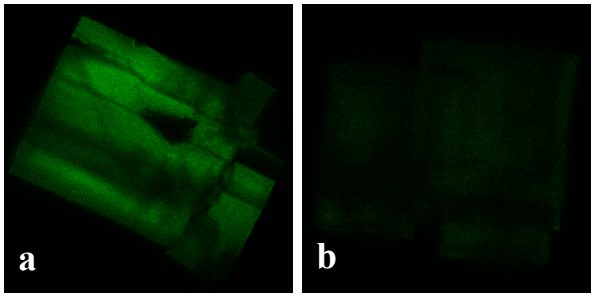



**Figure S19. Confocal laser scanning micrographs of two individual FITC@IRMOF-3 crystals, DT=700V.** Crystal S19-a and S19-b show higher amount of FITC captured inside IRMOF-3-mc comparing with the crystals with good crystallization quality. The defects inside crystal S19-a increased the chance of FITC encapsulation.

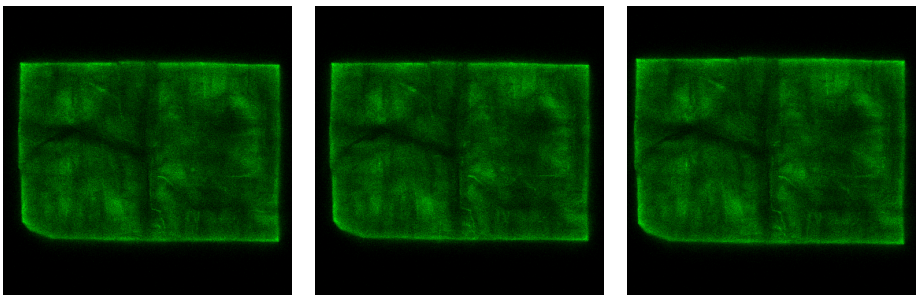


**Figure S21. Confocal laser scanning micrographs of FITC@IRMOF-1-mc prepared by the coprecipitation method, three slices from top down each in about 35 μm distance. Detector (photomultiplier tube) voltage=600V**.

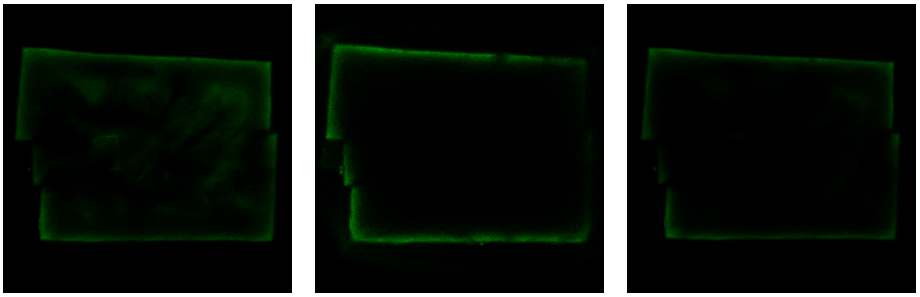


**Figure S21. Confocal laser scanning micrographs of FITC@IRMOF-1-mc prepared by the diffusion method, three slices from top down each in about 35 μm distance. Detector (photomultiplier tube) voltage=600V**. Some FITC adsorption to the external surface of IRMOF-1 similar to the IRMOF-3 case.