Electronic Supplementary Information

Bio-functionalization of metal-organic frameworks by covalent protein conjugation

Suhyun Jung, Youngmee Kim, Sung-Jin Kim, Tae-Hwan Kwon, Seong Huh*

and Seongsoon Park*

Experimental Methods

Chemicals, buffers, and CAL-B were purchased from TCI or Sigma-Aldrich. The Ni-NTA agarose resin was purchased from QIAGEN.

Synthesis of the compound 1D: A mixture of $In(NO_3)_3 \cdot xH_2O$ (0.301 g, 1.0 mmol) and 1,4-phenylenediacetic acid (0.388 g, 2.0 mmol) in 20 mL *N*,*N*-diethylformamide (DEF) was heated in a Teflon-lined high pressure bomb at 150 ºC for 72 h. Colorless needles were separated by filtration and washed with DEF and ethanol. The crystals were air-dried at ambient conditions. X-ray quality needles were chosen from the mother liquor.

Single-Crystal Structural Analysis of 1D-MOF (1): Data were collected on a Bruker SMART APX diffractometer using Mo-Kα radiation (λ = 0.71073 Å). The data were obtained at 150-170(2) K and refined by full-matrix least-squares refinements on F^2 using

all data with SHELXTL programs. The crystallographic data are listed in Table S1. Selected bond lengths and angles are listed in Table S2.

Expression and purification of the EGFP: The EGFP proteins were expressed and purified according to the literature (*S1*).

Activation of 1D-MOF: The 1D-compound (100 mg) was suspended in MES buffer (5 mL, 0.1 M, pH 5.0) and EDC (100 mg) was added. The mixture was then shaken for 20 min and centrifuged. After the supernatant being decanted, the activated 1D was washed three times with PBS buffer (5 mL, 0.1 M, pH 7.3) and resuspended in PBS buffer (1 mL).

Activation of 2D- and 3D-MOF: The 2D- and 3D-MOFs (100 mg) were suspended in a solution (10 mL) of 1% w/v hexanediamine and 1% w/v DCC in dichloromethane and shaken for 4 hr at 10 ºC. The activated compounds were washed with dichloromethane (10 mL), acetone (10 mL), and ice-cold water (10 mL), and resuspended in PBS buffer (1 mL).

Conjugation of proteins with the activated 1D-, 2D-, and 3D-MOF: The above suspension in PBS buffer (100 μL) and protein solution (100 μL, 1 mg mL^{-1}) were mixed and incubated at 4 ºC for 1 h (in the cases of 1D- and 3D-MOFs) or 16 h (in the case of 2D-MOF). The protein-conjugated 1D-, 2D- and 3D-MOFs were washed three times with PBS buffer (1 mL) and air-dried. The amount of the proteins in the solution was determined by Bio-Rad Protein Assay kit (Bio-Rad) according to the manufacturer's instructions. The

amount of the conjugated proteins to MOFs was estimated by the difference of the amount of protein in the supernatant before and after incubation.

Conjugation of EGFP with the CAL-B-conjugated 3D-MOF: EDC (10 mg) was added to an EGFP solution (100 μL, 3.4 mg mL^{-1} in 10 mM of PBS buffer, pH 7.3). The solution was incubated overnight at 4 ºC. The CAL-B-conjugated IRMOF-3 was added to the activated EGFP solution. The mixture was incubated at 4 °C for 1 h. The CAL-B and EGFP-conjugated 3D-MOF was washed three times with PBS buffer (1 mL, 10 mM, pH 7.3).

Physical adsorption of CAL-B to the MOFs without EDC- or DCC-activation: The MOFs (20 mg) was suspended in PBS buffer (100 μL, pH 7.3, 10 mM). A CAL-B solution (100 μL, 1mg/mL in PBS buffer) was added and incubated at 4 °C for 1 h (1D- and 3D-MOFs) or 16 h (2D-MOFs). The MOFs were washed three times with PBS buffer (1 mL) and air-dried. The amount of adsorbed CAL-B to MOFs was estimated by the difference in absorbance (ε = 41,285 M^{-1} cm^{-1} calculated with tools at Swiss Prot Expasy, http://ca.expasy.org/tools/protparam.html) of the supernatant at 280 nm, before and after incubation.

Physical adsorption of CAL-B after treatment of the activated MOFs by *n*-butylamine: The MOFs (20 mg) was suspended in a solution (1 mL) of hexanediamine (1% w/v) and DCC (1% w/v) in dichloromethane and shaken for 4 hr at 10 °C. The activated MOFs were washed with dichloromethane (1 mL), acetone (1 mL), and ice-cold water (1 mL), and resuspended in PBS buffer (100 μL, pH 7.3, 10 mM). An *n*-butylamine solution (50 μL, 1mg/mL in PBS buffer) was added to the activated MOF suspension and

incubated at 4 °C for 1 h (1D- and 3D-MOFs) or 16 h (2D-MOFs). The MOFs were washed three times with PBS buffer (1 mL). The protein solution (100 μL, 1mg/mL in PBS buffer) was added and incubated at 4 °C for 1 h (1D- and 3D-MOFs) or 16 h (2D-MOFs). The MOFs were washed three times with PBS buffer (1 mL) and air-dried. The amount of CAL-B binding to MOFs was estimated by the described above.

Spectroscopic Techniques: The emission spectra with set of λ_{ex} = 488 nm were recorded in the wavelength range of 450–650 nm with an LS 55 Luminescence Spectrometer. The slit width for excitation and emission was 5.0 nm and the scan speed was 100 nm min^{-1}.

Confocal Laser Scanning Microscope (CLSM): CLSM images of the EGFP-coated MOFs were obtained by using a Zeiss LSM 700 system. Green channel images were obtained with an excitation wavelength of 488 nm.

X-ray Powder Diffraction (PXRD): The samples for PXRD analysis were prepared as usual techniques. The crystals were grounded, mounted on the XRD holder and analyzed. PXRD patterns were obtained by using a Bruker D8 Focus with an X-ray tube with a Cu target.

Nitrogen Sorption Analysis: The N_2 sorption analysis was performed on a Belsorp-miniII (BEL Japan) at 77 K. The fresh as-prepared 3D-MOF (IRMOF-3) crystals immediately soaked in $CHCl_3$ in a screw-capped vial were shaken for two days. The $CHCl_3$-exchanged sample was dried at 393 K under high vacuum for 2 h before measurements. The CALB-

3D-MOF was also dried at 393 K under the same condition.

Measurement of catalytic activities of the CAL-B-conjugated MOFs toward the transesterification of (±)-1-phenylethanol: The CAL-B-conjugated MOFs or free CAL-B powders were mixed with (±)-1-phenylethanol (1 mmol) and vinyl acetate (1 mmol) in isopropylether (5 mL). The samples (100 μL) were retrieved with 30-min intervals for 420 min and analyzed by a GC with a chiral column (Cyclosil-B 30 m × 90.25 mm). The reactions were finished before 5% conversion reached in order to obtain the initial reaction rates. After the reaction was completed, the CAL-B-conjugated MOFs were recovered by centrifugation and washed three times with isopropylether. The recovered CAL-B-conjugated MOFs were used for the next run under the same reaction condition. The reactions were carried out three times. The GC condition: initial column temperature 80 ºC for 10 min, ramp up to 120 ºC at a rate of 2.5 ºC min^{-1} and then held at 120 ºC for 10 min.

Supporting Tables and Figures

Table S1. Crystal data and structure refinement for **1**.

Empirical formula	C_{24} H_{28} In N O_8	
Formula weight	573.29	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	Cc	
Unit cell dimensions	a = 19.192(4) Å	α = 90.00°
	b = 8.7720(18) Å	β = 120.71(3) °
	c = 16.666(3) Å	γ = 90.00°
Volume	2412.3(8) $Å^3$	
Z	4	
Density (calculated)	1.579 Mg/m^3	
Absorption coefficient	1.029 mm^{-1}	
F(000)	1168	
Crystal size	0.15 x 0.05 x 0.05 mm^3	
Theta range for data collection	2.47 to 25.996.	
Index ranges	$-17 \leq h \leq 23$, $-10 \leq k \leq 9$, $-20 \leq l \leq 20$	
Reflections collected	6523	
Independent reflections	3280 [R(int) = 0.0358]	
Completeness to theta = 25.99	99.7 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3280 / 2 / 309	
Goodness-of-fit on F^2	0.991	
Final R indices [I>2sigma(I)]	R_1 = 0.0382, wR_2 = 0.0764	
R indices (all data)	R_1 = 0.0650, wR_2 = 0.0847	
Absolute structure parameter	0.00	
Largest diff. peak and hole	0.474 and -0.365 e.$Å^{-3}$	

Table S2. Selected bond lengths [Å] and angles [°] for **1**.

In(1)-O(7)	2.250(11)
In(1)-O(3)	2.251(13)
In(1)-O(1)	2.278(14)
In(1)-O(5)	2.285(14)
In(1)-O(8)	2.291(12)
In(1)-O(2)	2.304(11)
In(1)-O(6)	2.306(14)
In(1)-O(4)	2.373(13)
In(1)-C(1)	2.524(16)
In(1)-C(3)	2.66(2)
In(1)-C(11)	2.660(10)
O(1)-C(1)	1.17(2)
O(8)-C(17)	1.30(2)
C(12)-C(13)	1.42(3)
C(12)-C(11)	1.50(2)
C(14)-C(15)	1.32(2)
C(14)-C(13)	1.33(2)
C(17)-O(7)	1.268(18)
C(17)-C(18)	1.40(2)
C(1)-O(2)	1.286(19)
C(1)-C(2)	1.65(2)
C(4)-C(3)	1.60(3)
C(4)-C(5)	1.61(2)
C(5)-C(10)	1.34(2)
C(5)-C(6)	1.43(2)
C(13)-C(19)	1.40(2)
C(8)-C(7)	1.37(2)
C(8)-C(9)	1.44(2)
C(8)-C(18)#1	1.52(3)
C(6)-C(7)	1.45(2)
C(2)-C(21)#1	1.50(3)

C(10)-C(9)	1.33(2)
O(5)-C(11)	1.15(2)
O(6)-C(11)	1.303(19)
O(4)-C(3)	1.28(2)
C(15)-C(21)	1.38(2)
C(3)-O(3)	1.15(2)
C(18)-C(8)#2	1.52(3)
C(21)-C(20)	1.32(3)
C(21)-C(2)#2	1.50(3)
C(20)-C(19)	1.42(3)
N(1S)-C(2S)	1.379(17)
N(1S)-C(3S)	1.40(2)
C(2S)-C(1S)	1.499(12)
C(4S)-C(3S)	1.62(3)

O(7)-In(1)-O(3)	131.5(4)
O(7)-In(1)-O(1)	87.1(5)
O(3)-In(1)-O(1)	138.0(5)
O(7)-In(1)-O(5)	131.8(4)
O(3)-In(1)-O(5)	82.4(4)
O(1)-In(1)-O(5)	80.7(5)
O(7)-In(1)-O(8)	56.1(4)
O(3)-In(1)-O(8)	98.3(5)
O(1)-In(1)-O(8)	90.80(19)
O(5)-In(1)-O(8)	167.5(5)
O(7)-In(1)-O(2)	130.87(16)
O(3)-In(1)-O(2)	81.3(5)
O(1)-In(1)-O(2)	58.0(5)
O(5)-In(1)-O(2)	79.4(5)
O(8)-In(1)-O(2)	88.3(5)
O(7)-In(1)-O(6)	79.9(5)
O(3)-In(1)-O(6)	108.4(2)
O(1)-In(1)-O(6)	92.2(5)

O(5)-In(1)-O(6)	54.5(5)
O(8)-In(1)-O(6)	135.6(5)
O(2)-In(1)-O(6)	129.6(5)
O(7)-In(1)-O(4)	81.5(5)
O(3)-In(1)-O(4)	54.3(4)
O(1)-In(1)-O(4)	167.7(5)
O(5)-In(1)-O(4)	103.6(3)
O(8)-In(1)-O(4)	86.6(6)
O(2)-In(1)-O(4)	133.8(4)
O(6)-In(1)-O(4)	81.4(5)
O(7)-In(1)-C(1)	109.2(5)
O(3)-In(1)-C(1)	111.2(6)
O(1)-In(1)-C(1)	27.5(6)
O(5)-In(1)-C(1)	78.4(5)
O(8)-In(1)-C(1)	89.8(5)
O(2)-In(1)-C(1)	30.4(5)
O(6)-In(1)-C(1)	111.5(6)
O(4)-In(1)-C(1)	164.1(5)
O(7)-In(1)-C(3)	108.4(5)
O(3)-In(1)-C(3)	25.4(5)
O(1)-In(1)-C(3)	163.4(5)
O(5)-In(1)-C(3)	92.8(5)
O(8)-In(1)-C(3)	93.0(5)
O(2)-In(1)-C(3)	106.0(5)
O(6)-In(1)-C(3)	96.4(6)
O(4)-In(1)-C(3)	28.9(5)
C(1)-In(1)-C(3)	136.2(6)
O(7)-In(1)-C(11)	108.8(4)
O(3)-In(1)-C(11)	92.2(5)
O(1)-In(1)-C(11)	88.6(5)
O(5)-In(1)-C(11)	25.5(5)
O(8)-In(1)-C(11)	164.8(5)
O(2)-In(1)-C(11)	104.1(5)

O(6)-In(1)-C(11)	29.3(5)
O(4)-In(1)-C(11)	90.7(5)
C(1)-In(1)-C(11)	96.6(5)
C(3)-In(1)-C(11)	91.9(2)
C(1)-O(1)-In(1)	88.1(11)
C(17)-O(8)-In(1)	94.0(10)
C(13)-C(12)-C(11)	124.8(16)
C(15)-C(14)-C(13)	127.2(16)
O(7)-C(17)-O(8)	112.3(16)
O(7)-C(17)-C(18)	127.3(14)
O(8)-C(17)-C(18)	119.6(15)
O(1)-C(1)-O(2)	129.6(17)
O(1)-C(1)-C(2)	121.7(17)
O(2)-C(1)-C(2)	108.2(15)
O(1)-C(1)-In(1)	64.4(11)
O(2)-C(1)-In(1)	65.3(8)
C(2)-C(1)-In(1)	169.8(12)
C(1)-O(2)-In(1)	84.3(10)
C(17)-O(7)-In(1)	97.0(10)
C(3)-C(4)-C(5)	105.4(13)
C(10)-C(5)-C(6)	119.7(16)
C(10)-C(5)-C(4)	125.7(14)
C(6)-C(5)-C(4)	114.6(15)
C(14)-C(13)-C(19)	116.1(17)
C(14)-C(13)-C(12)	127.8(17)
C(19)-C(13)-C(12)	116.0(18)
C(7)-C(8)-C(9)	118.1(17)
C(7)-C(8)-C(18)#1	118.2(17)
C(9)-C(8)-C(18)#1	123.5(15)
C(5)-C(6)-C(7)	114.6(16)
C(21)#1-C(2)-C(1)	121.2(15)
C(9)-C(10)-C(5)	126.2(17)
C(11)-O(5)-In(1)	95.8(10)

C(10)-C(9)-C(8)	117.5(16)
C(11)-O(6)-In(1)	90.6(10)
O(5)-C(11)-O(6)	117.8(12)
O(5)-C(11)-C(12)	131.0(15)
O(6)-C(11)-C(12)	110.6(15)
O(5)-C(11)-In(1)	58.7(8)
O(6)-C(11)-In(1)	60.1(7)
C(12)-C(11)-In(1)	170.2(12)
C(3)-O(4)-In(1)	88.0(12)
C(14)-C(15)-C(21)	119.4(16)
O(3)-C(3)-O(4)	120.3(19)
O(3)-C(3)-C(4)	131.3(17)
O(4)-C(3)-C(4)	108.5(17)
O(3)-C(3)-In(1)	57.1(11)
O(4)-C(3)-In(1)	63.2(10)
C(4)-C(3)-In(1)	171.6(12)
C(8)-C(7)-C(6)	123.2(16)
C(17)-C(18)-C(8)#2	108.4(15)
C(20)-C(21)-C(15)	115.3(17)
C(20)-C(21)-C(2)#2	119.1(17)
C(15)-C(21)-C(2)#2	125.1(18)
C(21)-C(20)-C(19)	126.1(18)
C(13)-C(19)-C(20)	115.5(17)
C(2S)-N(1S)-C(3S)	108.1(10)
N(1S)-C(2S)-C(1S)	111.1(11)
N(1S)-C(3S)-C(4S)	113.2(11)

Symmetry transformations used to generate equivalent atoms:

#1 x,-y,z+1/2 #2 x,-y,z-1/2

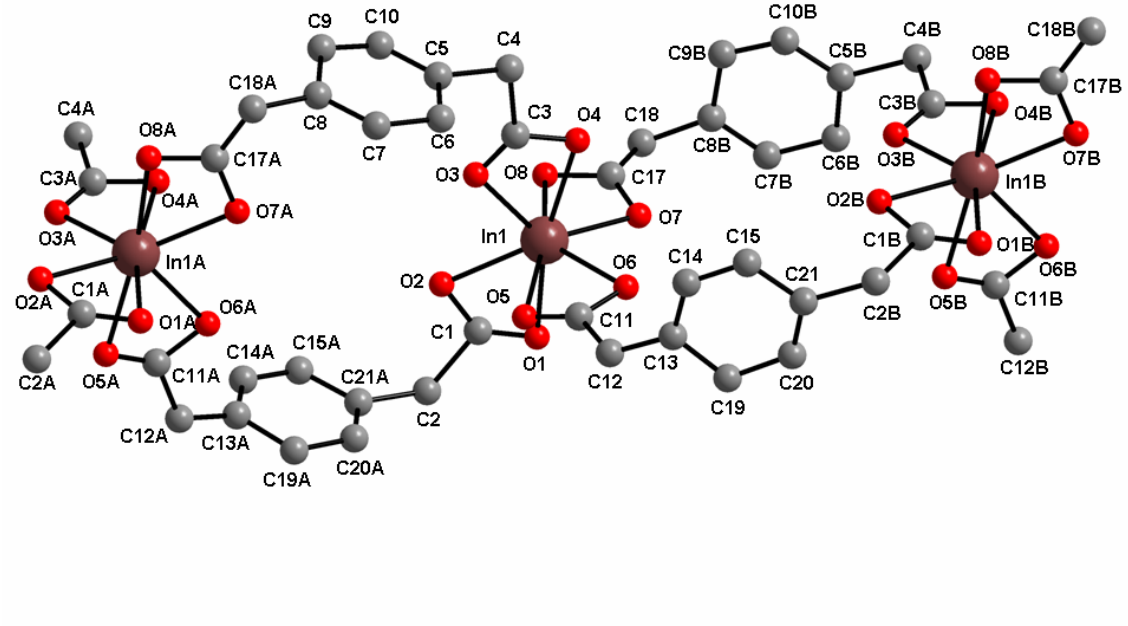

Figure S1. A perspective view of the 1D-MOF (**1**) indicating the pseudotetrahedral 8-coordinate In(III) centers. The $[H_2NEt_2]^+$ ions and hydrogen atoms are omitted for clarity. Symmetry operations: A (x, -y, 0.5+z) and B (x, -y, -0.5+z).

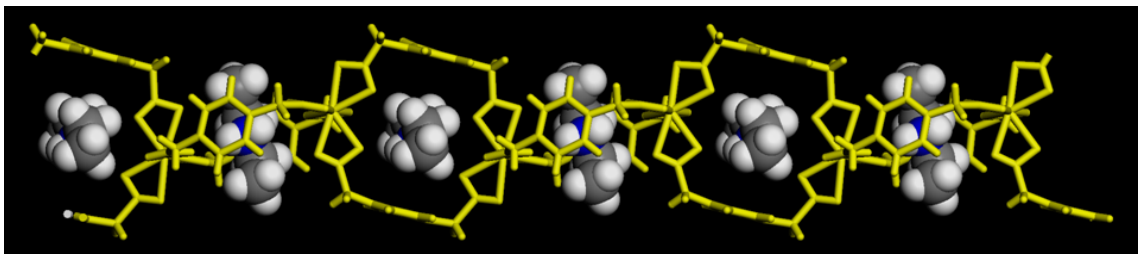

Figure S2. A view of the 1D-MOF (**1**) with the $[H_2NEt_2]^+$ ions. The $[H_2NEt_2]^+$ ions are shown in a CPK model.

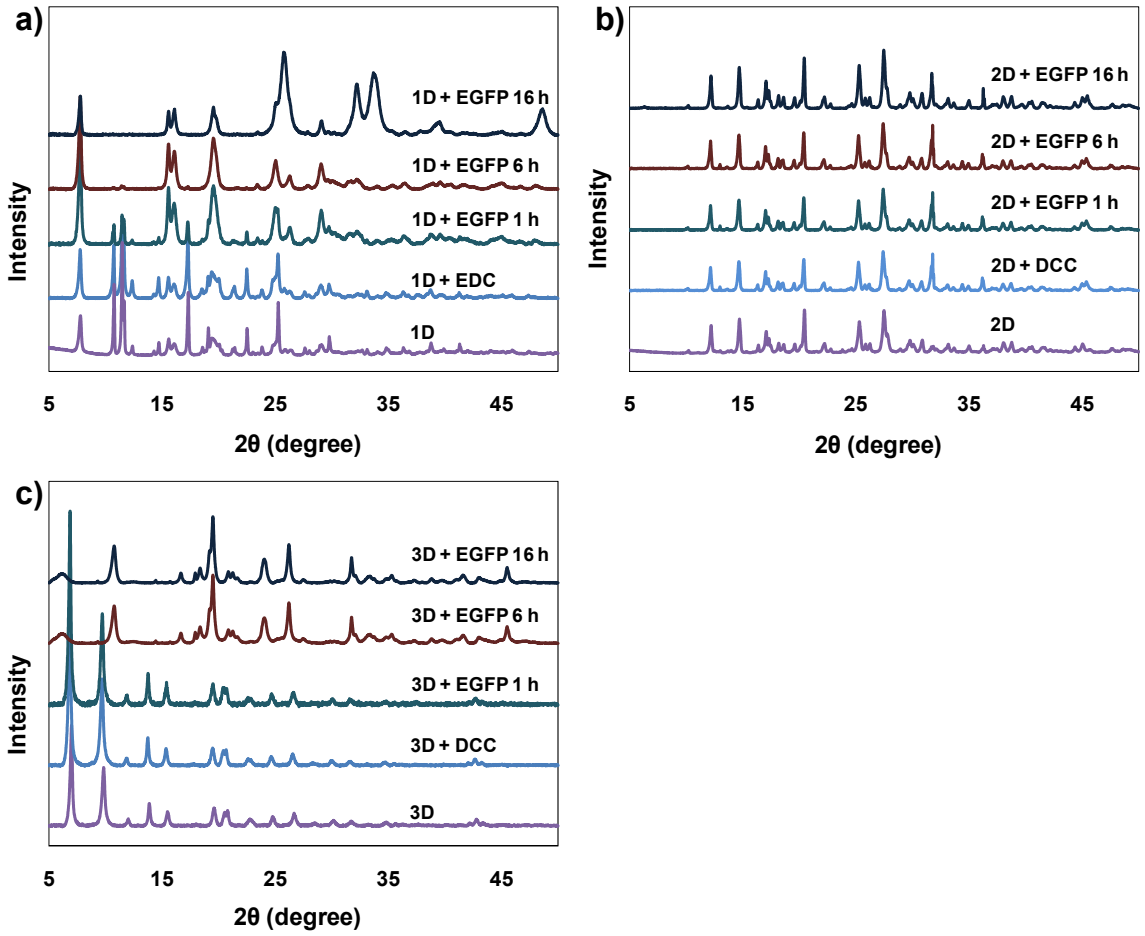

Figure S3. PXRD patterns of the 1D-MOF (a), 2D-MOF (b), and 3D-MOF (c), and the corresponding EGFP-conjugated 1D-, 2D-, and 3D-MOFs. The activation step by EDC or DCC did not alter the PXRD patterns for all MOFs.

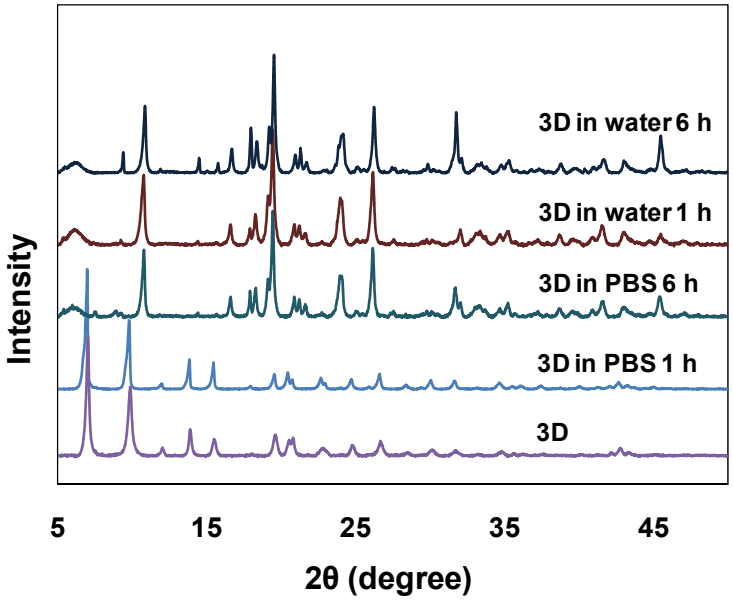

Figure S4. PXRD patterns before and after soaking the 3D-MOF in water and PBS buffer.

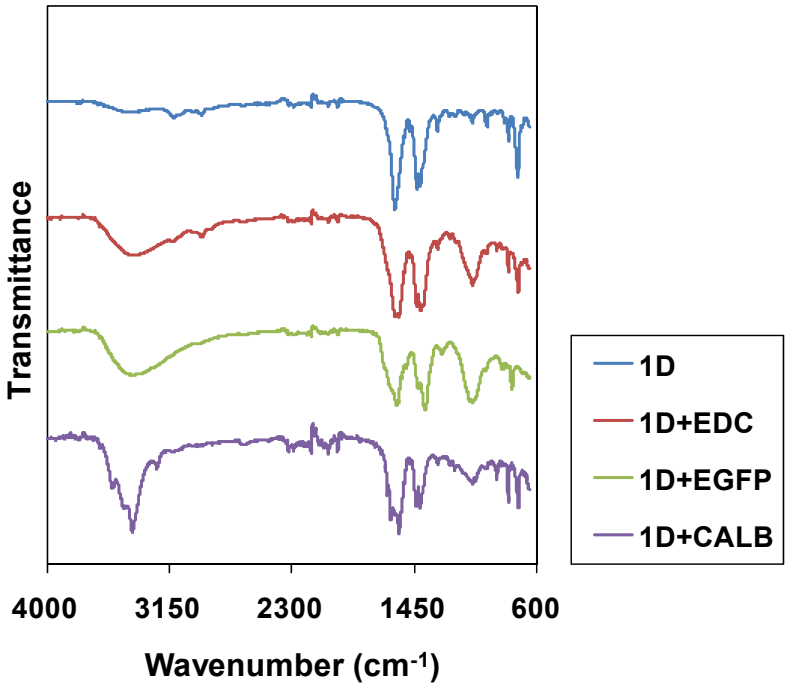

Figure S5. IR spectra of 1D-MOF and the conjugated 1D-MOFs.

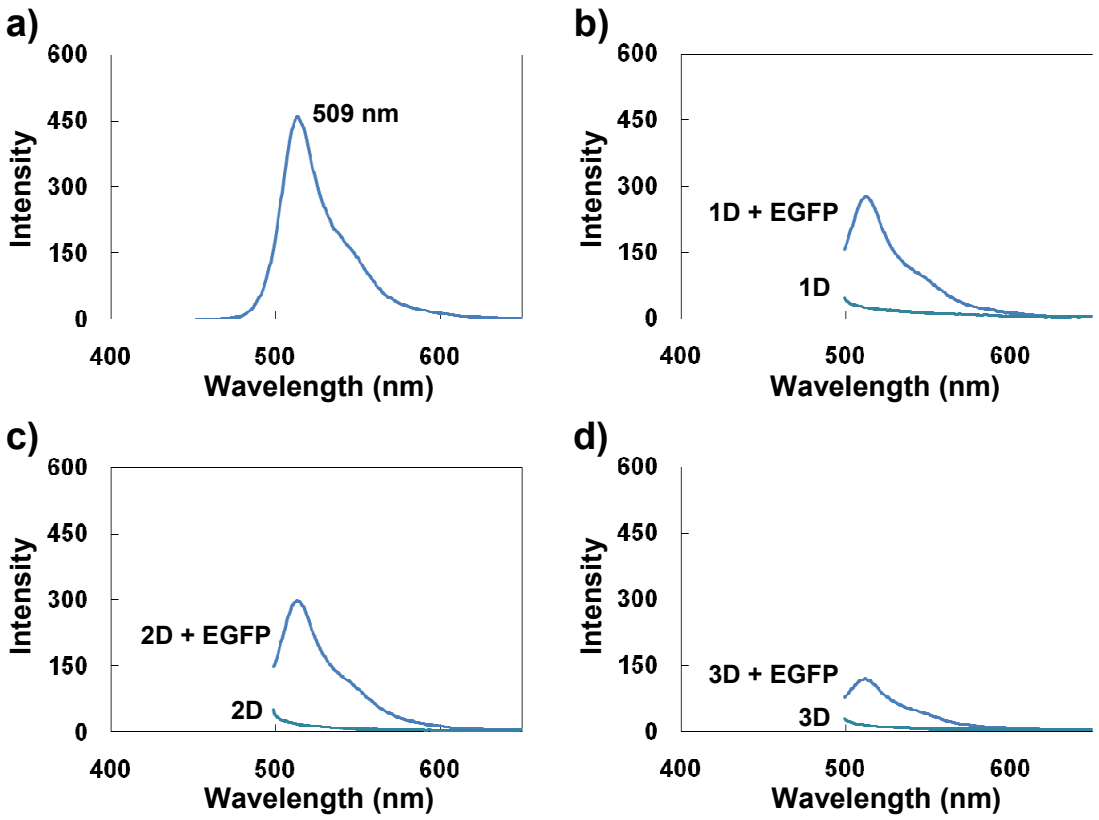

Figure S6. Emission spectrum: EGFP (a), 1D and 1D + EGFP (b), 2D and 2D + EGFP (c), and 3D and 3D + EGFP (d). The emission spectra were recorded under 488-nm excitation at room temperature. The characteristic maximum emission for EGFP is 509 nm.

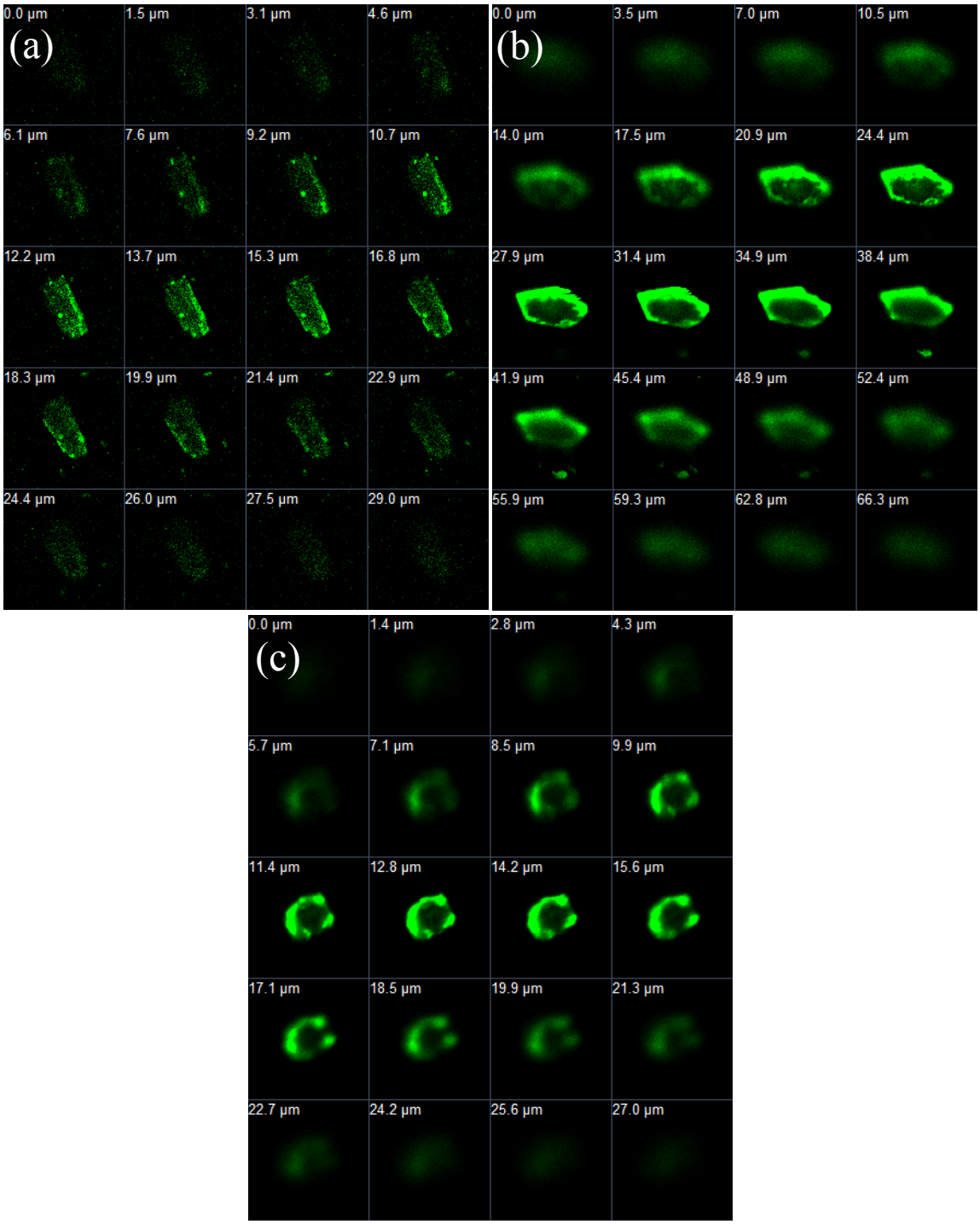

Figure S7. Z-stacked CLSM images of 1D + EGFP (a), 2D + EGFP (b), and 3D + EGFP (c). The excitation wavelength is 488 nm.

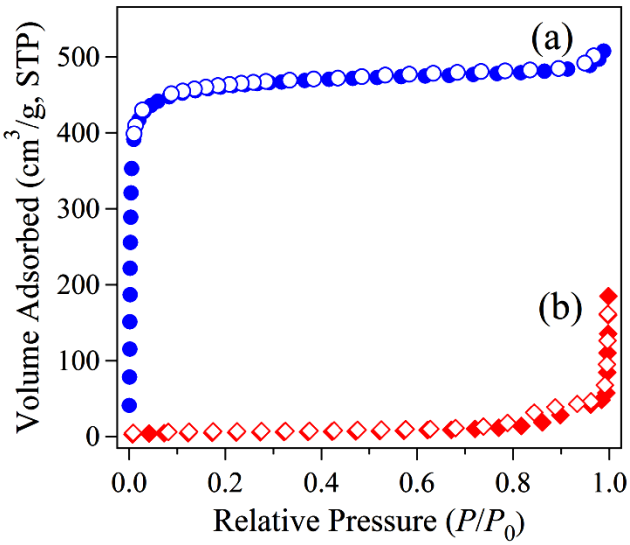

Figure S8. N_2 adsorption/desorption isotherms for the native 3D-MOF (a) and the CAL-B-3D-MOF (b) measured at 77 K. Both samples were activated at 393 K for 2 h.

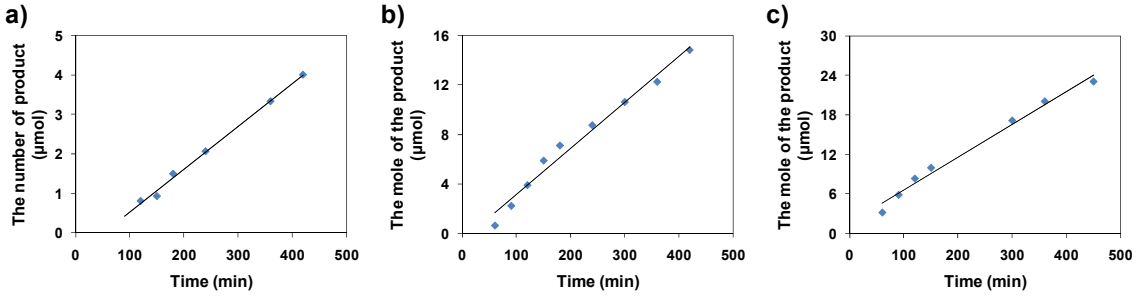

Figure S9. Measurements of the specific activities of CAL-B-conjugated MOFs: 1D-MOF + CAL-B (a), 2D-MOF + CAL-B (b), and 3D-MOF + CAL-B (c). Only a single measurement was shown as a representative example.

Table S3. The catalytic activity of physically adsorbed CAL-B on MOFs[a]

MOFs	The amount of physically adsorbed CAL-B on MOFs (mg g^{-1})	Specific activity (μmol min^{-1} mg^{-1})
1D-MOF	n.d.[b]	n.d.
1D + butyl amine	n.d	n.d.
2D-MOF	n.d	n.d.
2D + butyl amine	n.d	n.d.
3D-MOF	n.d	n.d.
3D + butyl amine	n.d	n.d.

[a] The reaction was same to that in Table 1, but no reaction occurs. [b] n.d. = not detected.

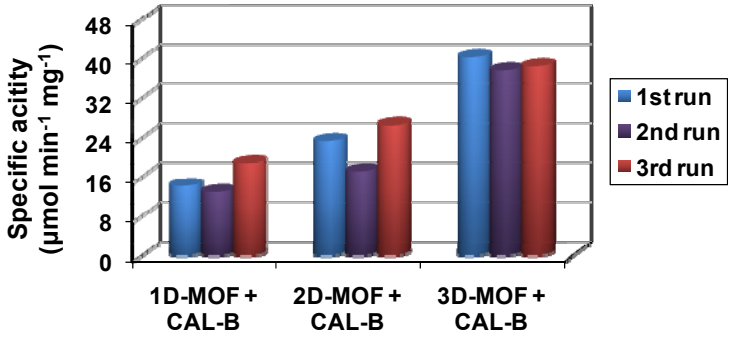

Figure S10. Recycling experiment results for the CAL-B-conjugated MOFs. The averaged values were shown in the Table 1.

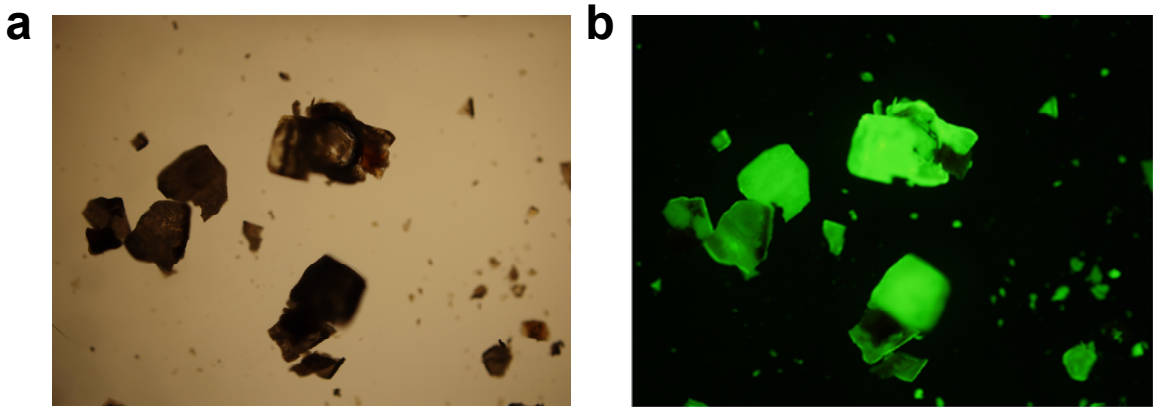

Figure S11. Microscopic images of dual protein decorated 3D-MOF: an optical microscopic image (a) and a fluorescence microscopic image (b).

Supporting references

(*S1*) Jung, S.; Huh, S.; Cheon, Y.-P.; Park, S. *Chem. Commun*. **2009**, 5003–5005.