Supplementary Information for:

Novel trypsin-FITC@MOF bioreactor efficiently catalyzes protein digestion

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Part I Synthesis and characterization of CYCU-4

1. **Synthesis of CYCU-4**

A reaction mixture of H_2SDC (0.097 g, 0.36 mmol), $Al(NO_3)_3 \cdot 9H_2O$ (0.176 g, 0.47 mmol), and N,N-diethylformamide (DEF) (10.0 ml) was stirred for 5 min at room temperature, forming a homogeneous solution. The solution was heated at 180 °C for 3 days. A pale-yellow powder was filtered off, washed with dimethylformamide (DMF), dried at 120 °C in an oven, and collected, yielding a product weighing 0.1947 g.

2. X-ray Powder Diffraction

Structural characterization was carried out with a Panalytical X'Pert Pro MPD diffractometer (Bragg-Brentano para-focusing geometry, reflection mode) with K-Alpha1 wavelength of 1.540598 Å and K-Alpha2 wavelength of 1.544426 Å operated at 40 kV and 30 mA in reflection geometry using flat plate samples. The measurements were performed from 2-50° 2 Theta with the step-scan mode with a step-width of 0.0021^o and a sampling time of 65s per step.

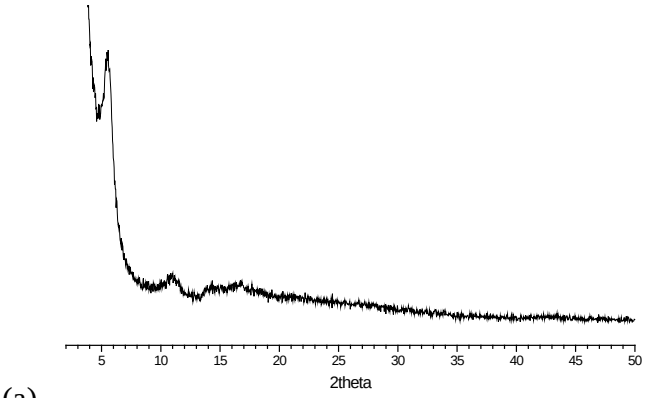
3. Structural Modeling and Powder X-Ray Diffraction Analysis.

The theoretical model of CYCU-4 was carried out using the Materials Studio 5.5.[1] Initially, only the Al atoms were included using the fractional coordinates found in MIL-68(V). The unit cell was elongated accordingly by the experimental lattice constants. Finally, the atoms of the 4,4'-stilbenedicarboxylate linker without hydrogen atoms were inserted. The geometry optimizations converged to give a final plausible crystal structure by adjusting the bond lengths of aluminum to oxygen atoms (bond angles of O-Al-O). The calculated and measured powder X-ray diffraction patterns were in good agreement (Fig. S1). The export SHELX res file was further examined by

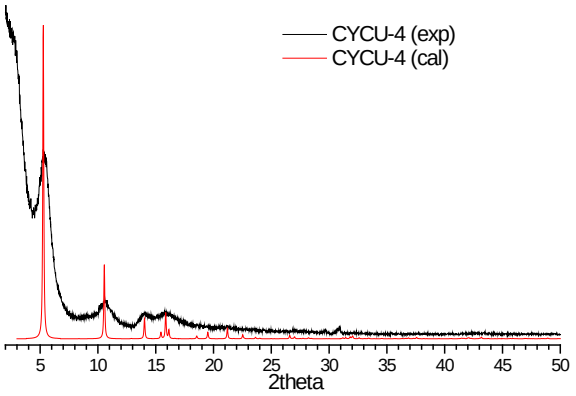
PLATON [2] software by the Addsym command. This would produce a new suitable res file for further use on the structure analysis. The models were used as a starting point for the Rietveld refinement.

The structure was solved from the powder XRD pattern of the activated sample, heated at 150 ^{o}C, to prevent re-absorption of water. The lab powder X-ray diffraction was measured for further considerations. Extractions of the peak positions, pattern indexing and Rietveld refinements were carried out with the GSAS program.[3] For CYCU-4, due to the highly broadened peaks displayed in the powder XRD, the *Imma* space group, as reported in MIL-53 and DUT-5, was chosen to solve the structure. The pattern could be successfully indexed as an orthorhombic system with a lattice constant of a=28.74, b=6.447, and c=19.04 Å. The final Rietveld plot corresponding to the crystal structure model and profile factors were display in Fig. S1. table S1 and table S2 list the crystallographic data and atomic coordinates.

4.



(a)



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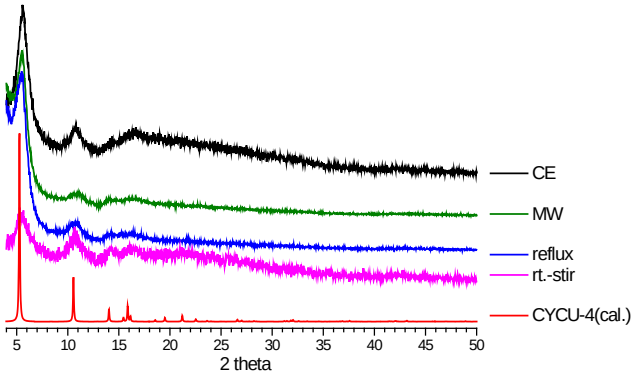

(c)

Figure S1: Powder XRD patterns of: (a) as-synthesized CYCU-4, (b) desolvated CYCU-4; (c) desolvated CYCU-4 under different reaction method (conventional electric (CE), microwave (MW), reflux at 180^{o}C and stir at room temperature (rt-stir).

5.

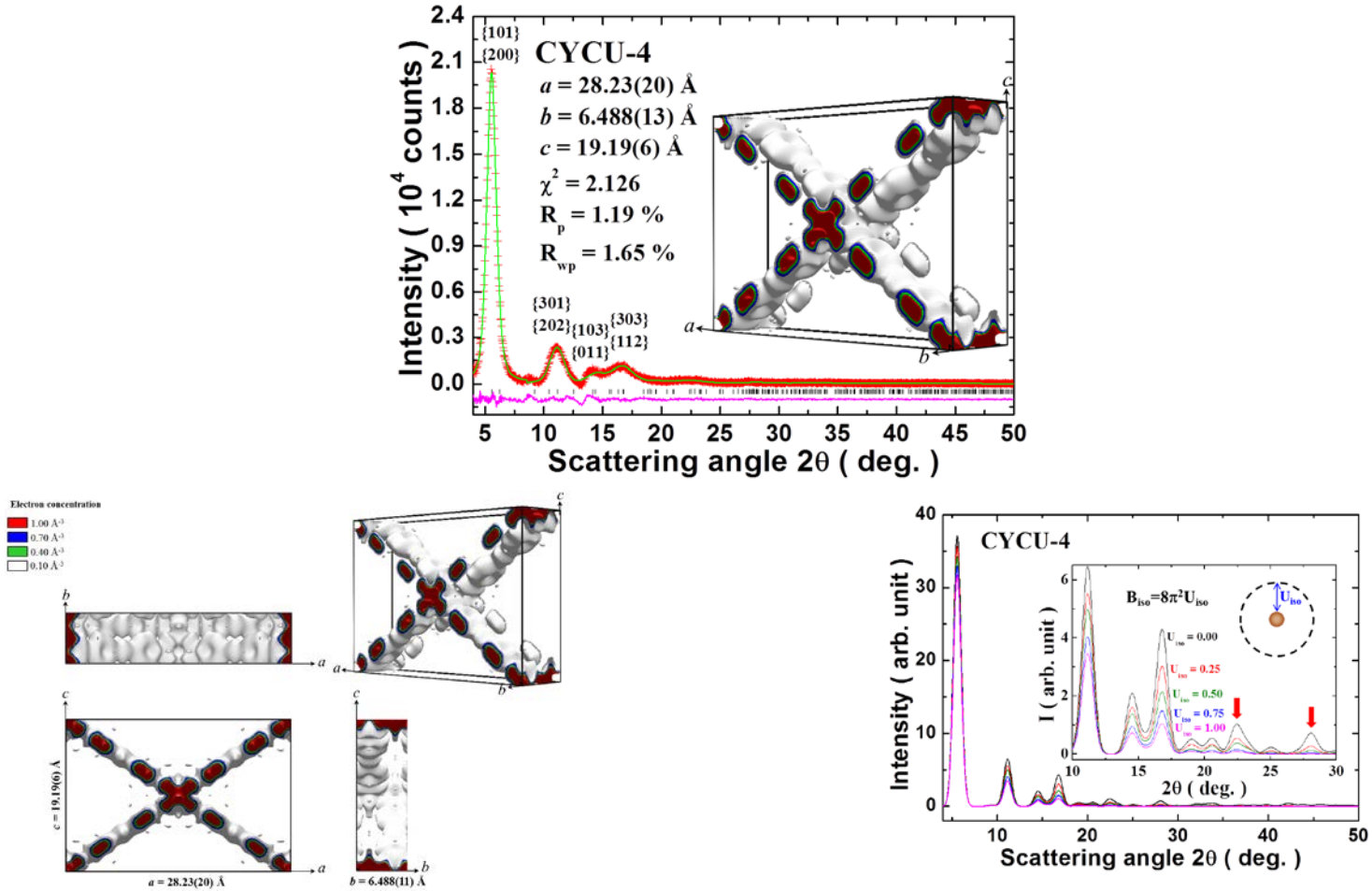

Figure S2. The plots of electron density from X-ray powder diffraction pattern of CYCU-4.

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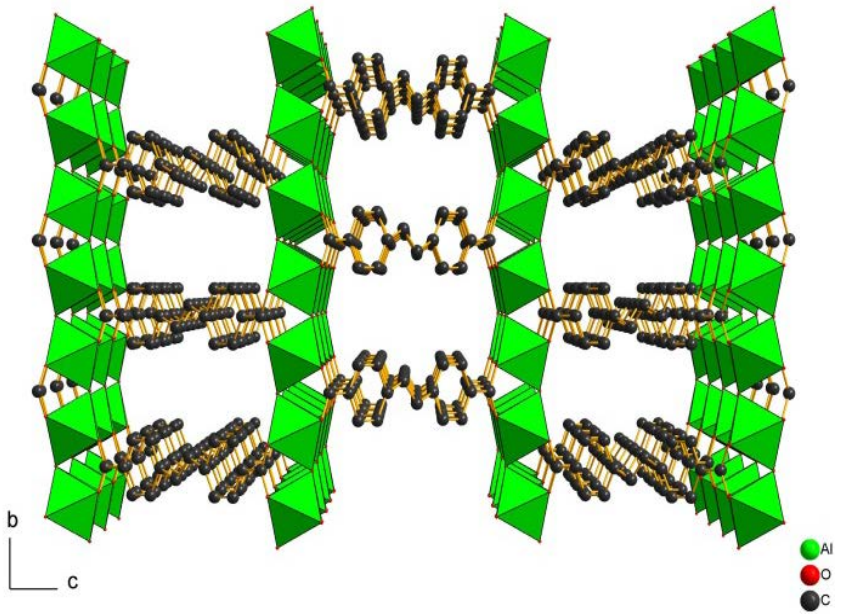
(a)



(b)

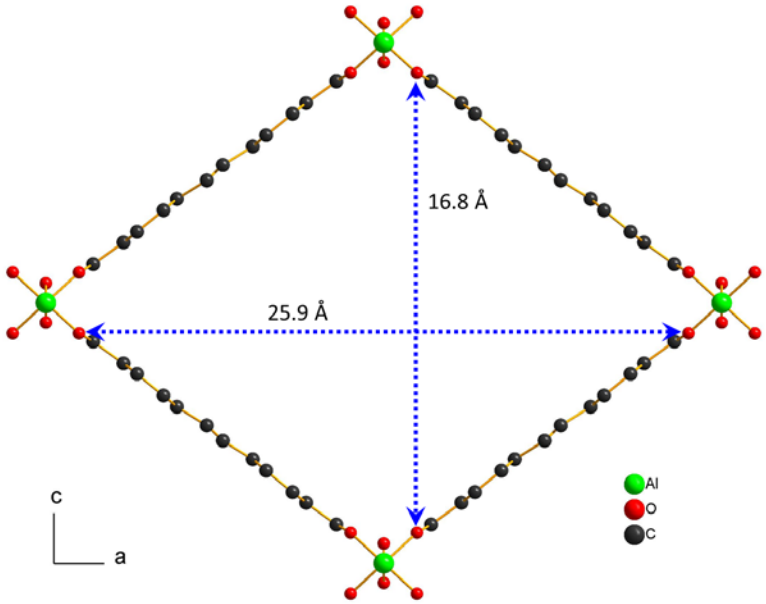


Figure S3. (a) MOF structure with the cornered-sharing 1D inorganic chain (b) pore sizes of CYCU-4.

7.

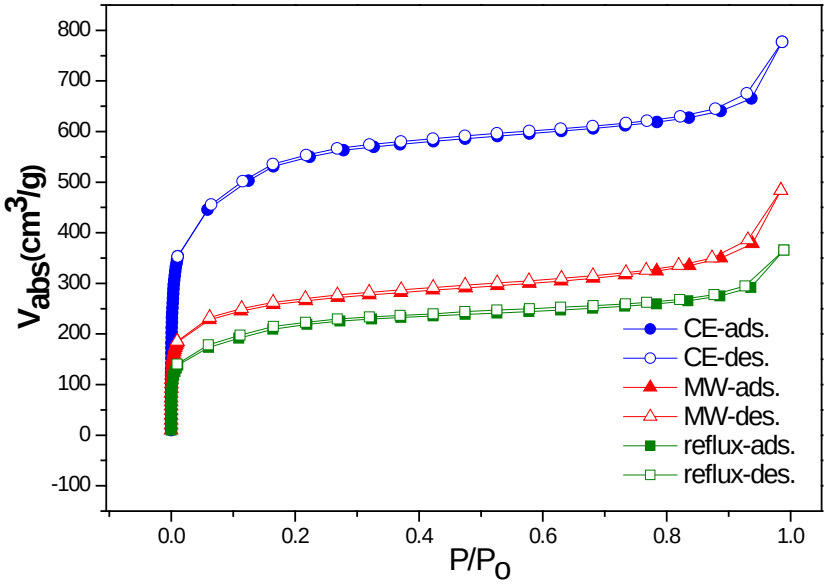


Figure S4.Nitrogen adsorption and desorption isotherms (77 K) of CYCU-4 prepared by different reaction methods (solid circles, adsorption; open circles, desorption).

8.

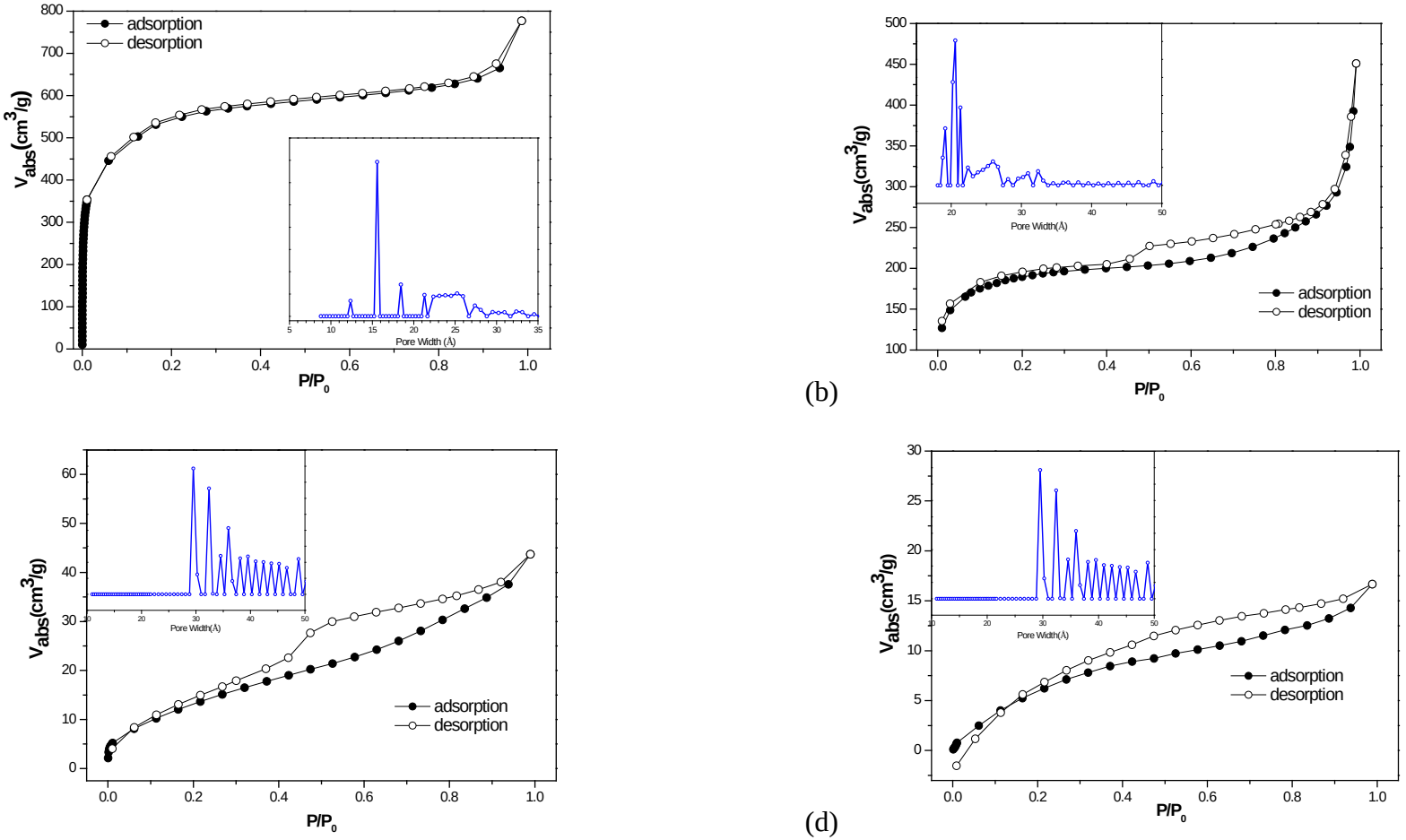


Figure S5. The nitrogen adsorption and desorption isotherms (77 K) and pore size distributions (DFT) of (a) native CYCU-4, (b) CYCU-4 after immersion in water and washing with water and hot DMF, (c) CYCU-4 after immersion in FITC solution and (d) CYCU-4 after immersion in trypsin-FITC solution.

9.

Table S1. Summary of synthesis conditions and products

Al^{3+} source	Solvent	Time (d)	Temp. (^oC)	Additives	method	Product
$Al(NO_3)_3$	DEF	1d	180	-	CE[a]	CYCU-4
$Al(NO_3)_3$	DEF	1d	180	-	reflux[b]	CYCU-4
$Al(NO_3)_3$	DEF	30min	180	-	MW[c]	CYCU-4
$Al(NO_3)_3$	DEF	1d	25	-	stir[d]	Unknown
						"Al(III)+H_2SDC"

[a] conventional electric (CE)
[b] reflux in 180 ^oC
[c] microwave (MW)
[d] stir in room temperature (rt-stir)
*DEF , N,N-Diethylformamide

10.

Table S2. Results and relevant information for the Rietveld refinement of CYCU-4.

	CYCU-4
Formula	$Al_4O_{20}C_{64}H_{44}$
Formula weight	1240.2
Temperature (K)	298
Wavelength (Å)	1.54056
Pattern range (°, 2θ)	3– 50
Space group	*Imma* (No. 74)
a (Å)	28.23(20)
b (Å)	6.488(13)
c (Å)	19.19(6)
V (Å^3)	3515.3(44)
Z	8
χ^2	2.126
R_p	0.0119
R_{wp}	0.0165

11.

Table S3. Atomic coordinates and equivalent isotropic displacement parameters (Å^3).

	x	y	z	U_{iso}	Occupancy
A1	0.0000	0.0000	0.0000	0.1	1
O1	0.0000	-0.2500	-0.0377	0.1	1
O2	-0.0490	-0.0826	0.0585	0.1	1
C1	-0.0701	-0.2500	0.0745	0.1	1
C2	-0.1148	-0.2500	0.1161	0.1	1
C3	-0.1349	-0.0694	0.1366	0.1	1
C4	-0.1740	-0.0694	0.1778	0.1	1
C5	-0.1939	-0.2500	0.1998	0.1	1
C6	-0.2381	-0.1767	0.2352	0.1	0.5

Part II Application of CYCU-4

1. Trypsin-FITC conjugation and immobilization onto MOFs.

A solution containing 50 µL of 6000 µg mL^{-1} TPCK- treated trypsin standard stock solution, 50µL 5mM FITC and 50 µL sodium carbonate buffer (100 mM) was vortex-mixed gently. The homogenous mixture was placed in a beaker with room temperature water and then microwave-heated for 2 min (180 W, SAMPO domestic microwave oven model RE-1002SM). The trypsin-FITC was immobilized onto the native MOFs (1mg) by suspending in a 100 µL trypsin-FITC solution, gently vortexed at RT for 30 min. The produced trypsin immobilized MOFs (trypsin-FITC@MOF) were washed three times with 100 µL phosphate buffer prior to further digestion and submission to other tests.

2. BSA denaturation process

The denaturation process followed the procedure of Kinter, et. al.[4] but with slight modification. A 10 mg of lyophilized BSA protein was resuspended in a 1mL aqueous solution composed of urea (6 M) and Tris buffer (100 mM). A 5 µL of dithiothreitol (DTT) reducing reagent (200 mM) was added to a 100 μL aliquot of the BSA solution (10 mg/mL) in a 1.5 mL plastic micro-centrifuge tube, and then vortex-mixed gently. The protein mixture was allowed to stand at RT for 1 h. A 20 µL of the alkylating reagent, iodoacetamide (IAA, 200 mM), was added into the above solution with a gentle vortex, and then the alkylation process proceeded at RT in the dark for 1 h. To consume the unreacted IAA, another 20 µL of DTT reducing agent was added to the resulting solution with gentle vortex mixing, and the reaction was allowed to stand at RT for 1 h. The resulting solution was mixed with 775 µL of D.I. water in order to reduce the urea

concentration to ~ 0.6 M, at which the trypsin can retain its activity.

3. BSA digestion via trypsin-FITC@MOFs.

The denatured BSA solution (100 μL) was added into the trypsin-FITC@MOF biocatalysts with gentle vortex- mixing, and then an ultrasonic-assisted digestion reaction was carried out for 2 min. The peptide solution was separated from the solid MOF biocatalysts by 5-min centrifugation. An acetic acid solution (1μL of 17.4 M) was added to stop the digestion reaction. The resulting acidified supernatant was desalted using ZIP-TIP-C18 following the manufacturer's procedure. The resulting peptide solution was diluted with ammonium acetate solution (50 mM, pH 8.75) containing 5% ACN prior to nano-LC-MS^2 analysis.

4. Conditions for nano-LC-MS^2

A poly(stearyl methacrylate-divinyl benzene-vinylbenzyltrimethylammouium) (poly(SMA-DVB-VBTA)) monolithic separation column (70 cm length; 75μm I.D.) modified from the previous study of Hsu, et. al [5] was used as separation column for nano-LC-MS^2, which was equilibrated with 100% solvent A (A: 95% water, 5% ACN, 0.1% FA, v/v). BSA peptide digests were separated in a gradient mode (0-10 min, 100% A; 10-60 min, 100%-30% A; 60-65 min, 30%-0% A, with solvent B (B: 20% water, 80% ACN, 0.1% FA, v/v) at a flow rate of 250 nL/min). The 0.5 μL sample was injected via an autosampler (Dionex) with a 1μL sample loop. Nano-LC-ESI-MS^2 was performed on a UltiMate 3000 Nano-LC system (Dionex, Amsterdam, The Netherlands) coupled to the amazon SL mass spectrometer (Bruker-Daltonik, Germany) equipped with a nanoelectrospray ionization source (Bruker). All devices were controlled using HyStar

3.2 (BrukerDaltonik). A spray voltage of -1.5 kV was employed and the temperature of the transfer capillary was set to 250°C. MS data were acquired over 85 min in a data-dependent MS^2 mode. The full-scan mode in the ion trap analyzer covered the range of *m*/*z* 100-2200 at an enhanced resolution mode followed by product ion scans of the 2 most intense signals in the full-scan mass spectrum. Dynamic exclusion, with a duration of 60 s, was used to detect less abundant ions. MS^2 collision energy was set to 27%, and the fragment ions were detected at the ion trap analyzer. Peptide fragment fingerprint MS^2 analyses for protein identification were performed with BioTools 3.1 (BrukerDaltonik) using the Mascot database. Protein identification based on nano-LC-ESI-MS^2 experiments was achieved by conversion of raw data to mgf-files, using the Data analysis 4.0 software (BrukerDaltonik). The mgf-files were used to search the SwissProt database with BioTools 3.1 (BrukerDaltonik). MS^2 data were also included in the Mascot search.

5. Determination of trypsin activity and loading capacity of trypsin-FITC@CYCU-4

The activities of immobilized trypsin were measured by the hydrolysis of N-α-benzoyl-*DL*-arginine (BAPNA) in 10mM sodium phosphate buffer (pH 7.9). The resulted hydrolysis product, *p*-nitroaniline, was monitored by measuring the increase in the absorbance at 405 nm with a UV–Vis spectrophotometer. Herein, 500 μL 1mM BAPNA in10mM sodium phosphate buffer (pH 7.9) was added to the trypsin-FITC@CYCU-4 suspension. After 15 min, the trypsin-FITC@CYCU-4 was separated by centrifugation and the absorbance of the supernatant was measured at 405nm with a UV–Vis spectrophotometer.

The loading capacity of CYCU-4 for trypsin-FITC was evaluated by fluorescence emission method and the activity test method by the

BAPNA hydrolysis, respectively. First, the difference in the fluorescence emission intensity of trypsin-FITC solution before and after immobilization into CYCU-4 was used to obtain the concentration of trypsin-FITC adsorbed on CYCU-4. After calibration of solution volume (500μL) and initial trypsin concentration (2000 μg/mL) used, the loading capacity of trypsin-FITC@CYCU-4 was about 55.2 μg trypsin /mg MOF. For the activity test method, the UV absorbance of the hydrolysis product (p-nitroaniline) obtained from trypsin-FITC@CYCU-4 and free trypsin (2000 ppm trypsin in-solution) were compared, and the ratio of both activity tests were used to obtain an approximate loading capacity of around 63.2 μg trypsin /mg MOF, which was close to the results from the fluorescence method.

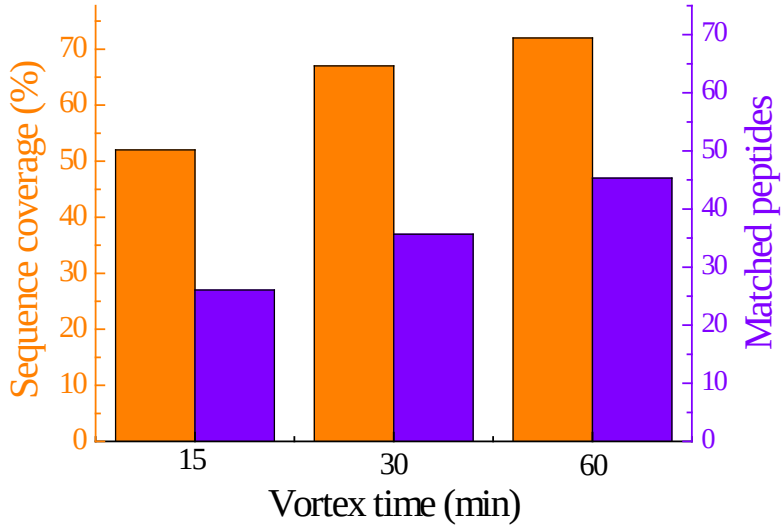


Figure S6 BSA digests from the trypsin-FITC@CYCU-4 bioreactor prepared with different vortex time to enhance trypsin-FITC absorption into CYCU-4.

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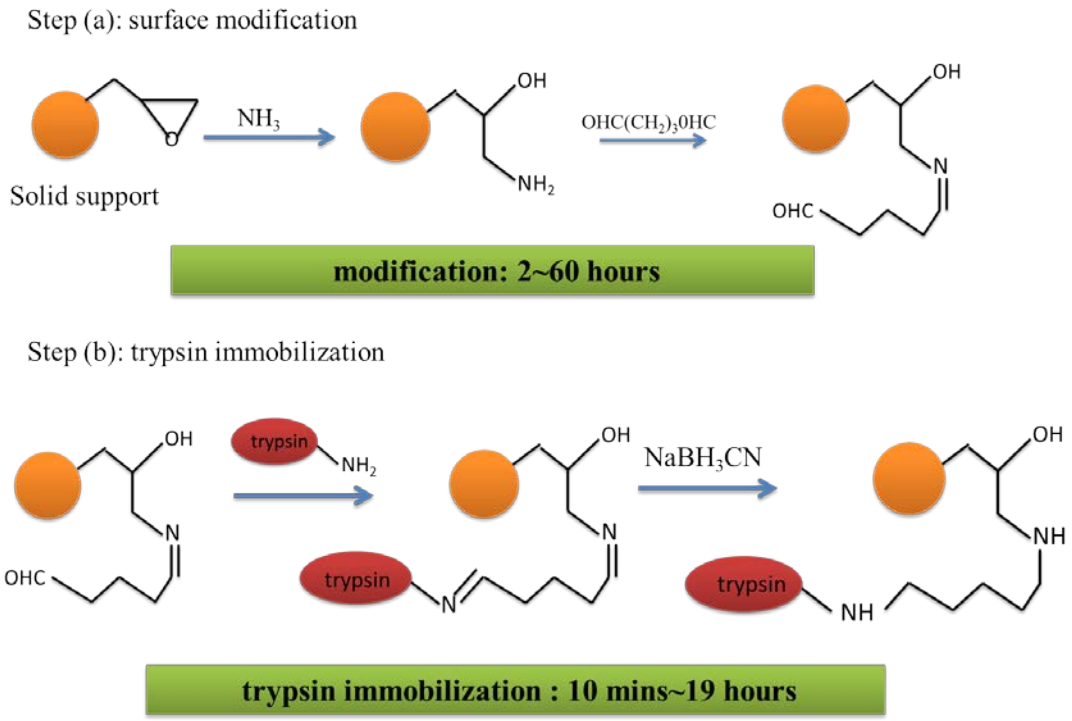

Figure S7. Conventional preparation procedures used to immobilize trypsin on solid supports [6-13].

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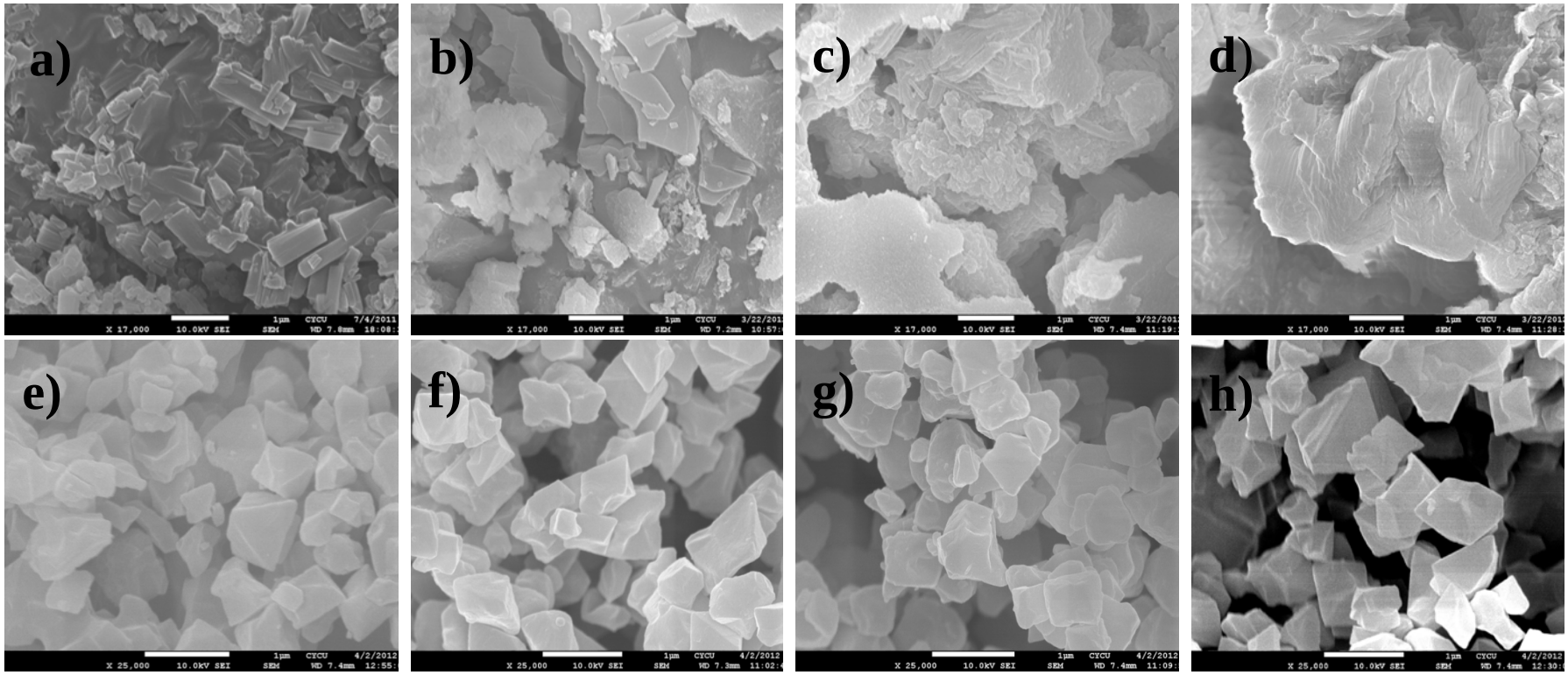
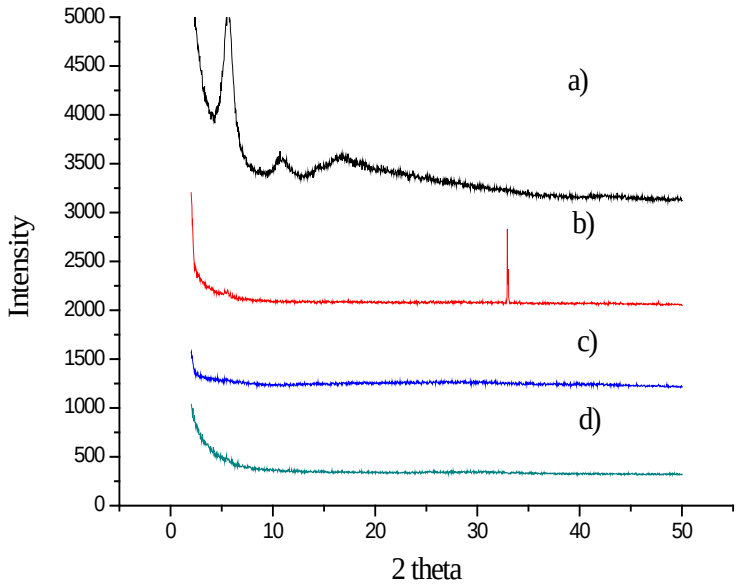


Figure S8 SEM images of a) as synthesized MOF, b) dried MOF after sodium carbonate buffer soaking, c) FITC@MOF, d) trypsin-FITC@ MOF biocatalysts. Top images (CYCU-4) and bottom images (MIL-101(Cr)).

9.

(A)



(B)

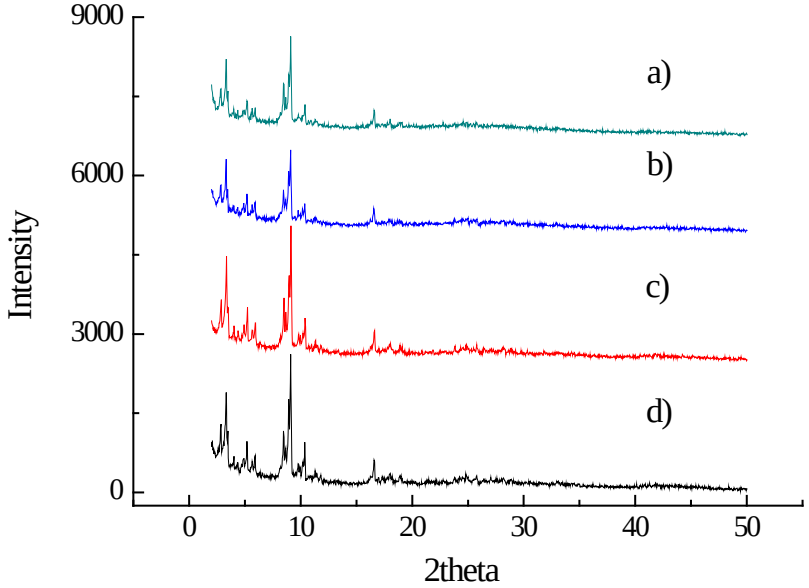


Fig.S9(A) Powder XRD patterns of a) native CYCU-4); b) sodium carbonate buffer immersed CYCU-4; c) FITC@ CYCU-4 ; d) trypsin-FITC@ CYCU-4, (B) a) native MIL-101(Cr); b) sodium carbonate buffer immersed MIL-101(Cr); c) FITC@ MIL-101(Cr) ; d) trypsin-FITC@MIL-101(Cr).

10.

Figure S10. 3D CLSM images of Trypsin-FITC@ CYCU-4 biocatalyst constructed based on a hundred slices chosen from the bottom to the top of about 83 um z-axis height. Linked to website at DOI: 10.1039/b000000x/

11.

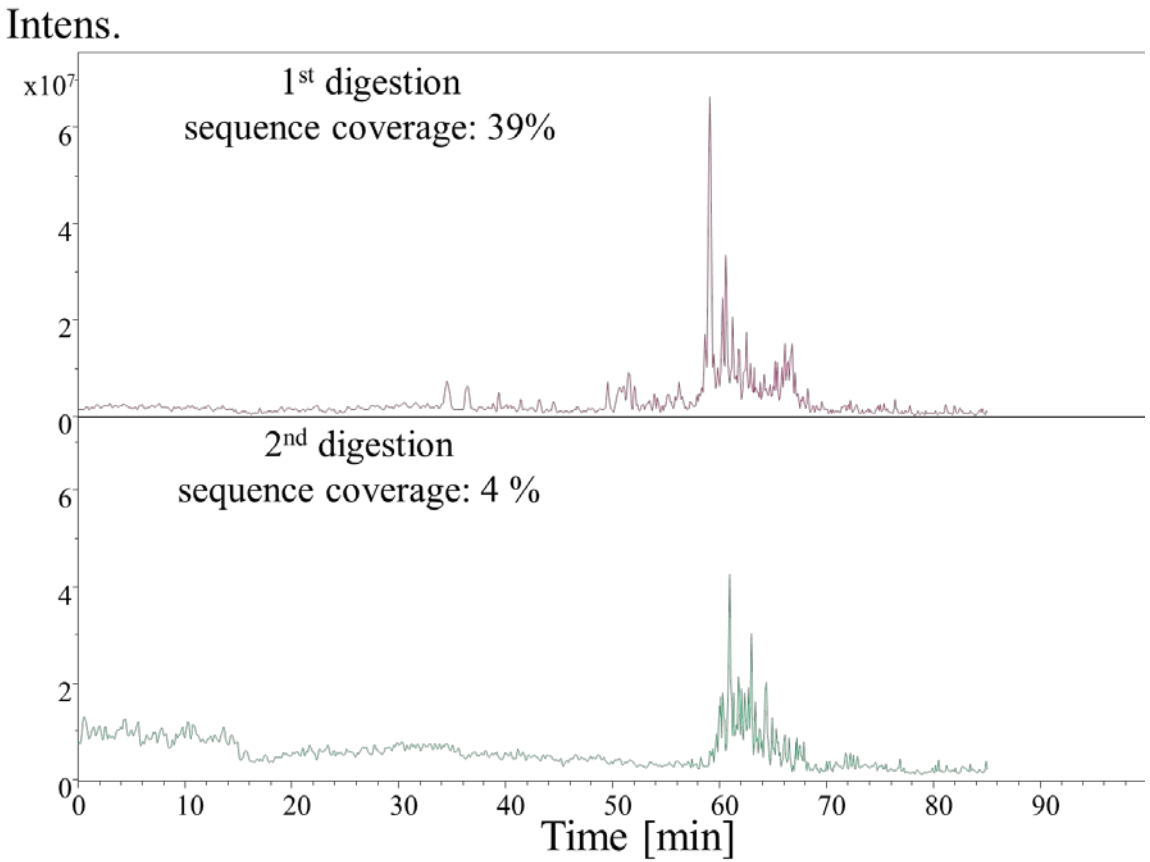


Figure S11. NanoLC-MS^2 chromatogram of BSA peptides digested by"Al(III) + H_2SDC" material (non-porous).

12.

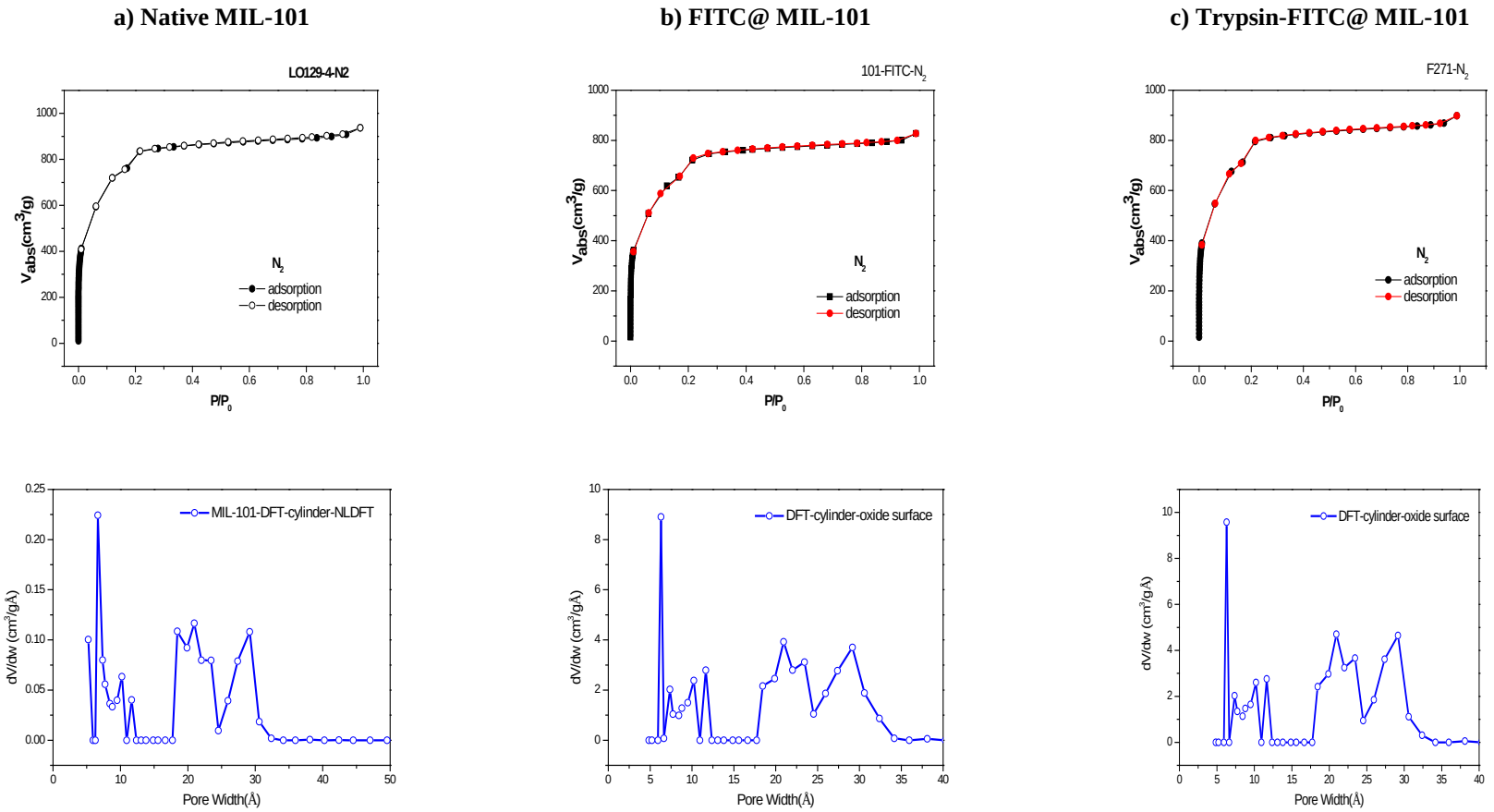


Figure S12. The nitrogen adsorption and desorption isotherms (77 K) and pore size distributions (DFT) of (a) native MIL-101, (b) MIL-101 after immersion in FITC solution and (c) MIL-101after immersion in trypsin-FITC solution.

13.

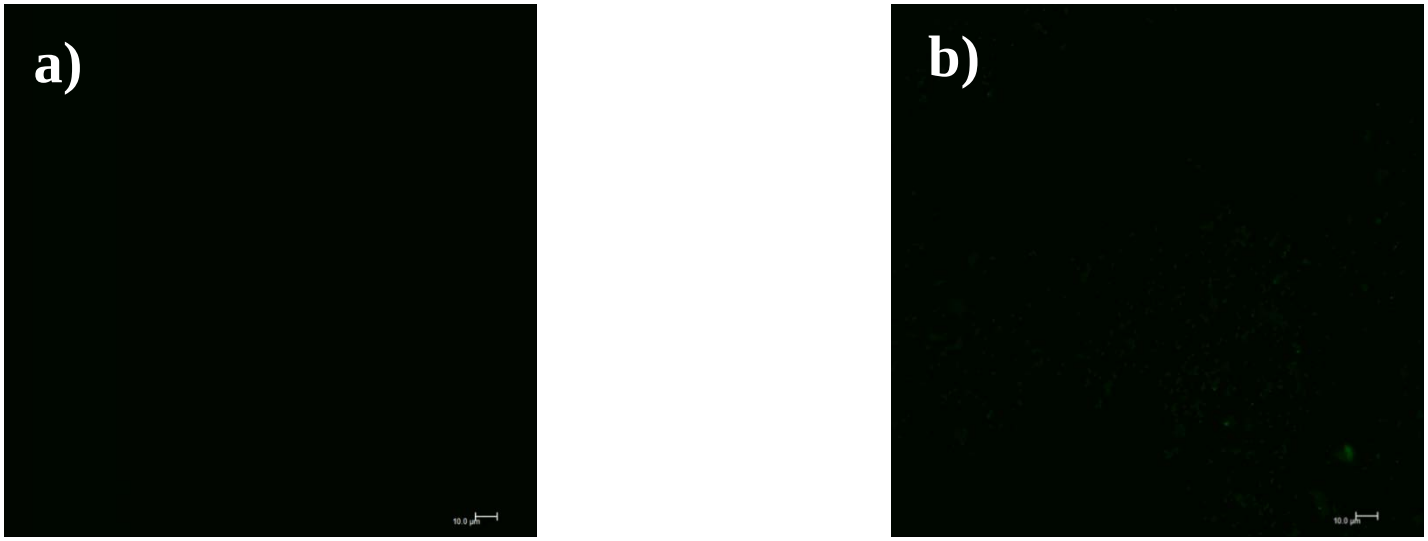


Figure S13. Fluorescent images of the MOFs. a) MIL-101 (Cr) . b) trypsin-FITC@ MIL-101 (Cr).

14.

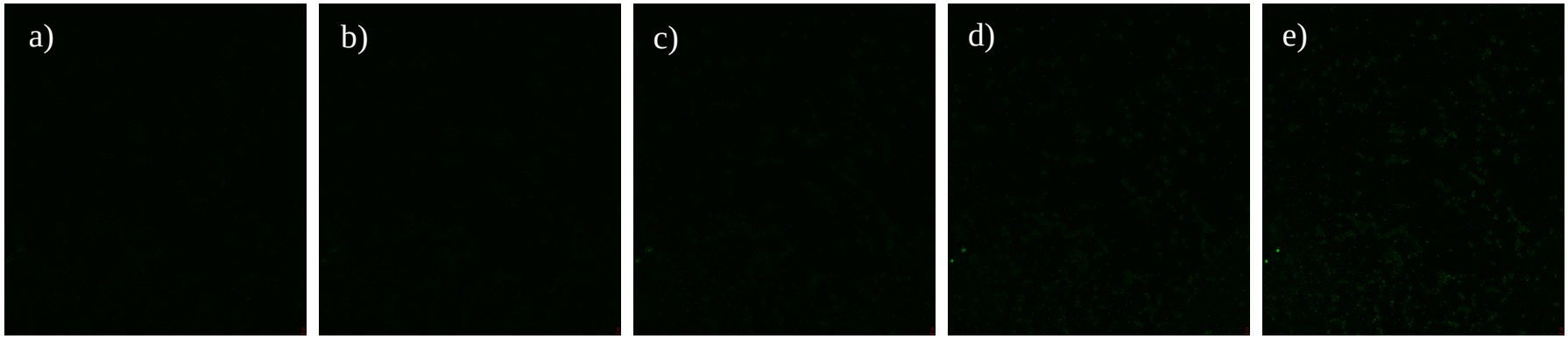


Figure S14 Confocal laser scanning micrographs of MOFs (a-e) trypsin-FITC@MIL-101(Cr). The slices chosen for (a)–(e) were about 83nm distance along z direction. For the CLSM, an excitation filter λex= 450–480 nm and long pass emission filter λem> 515 nm were chosen.

15.

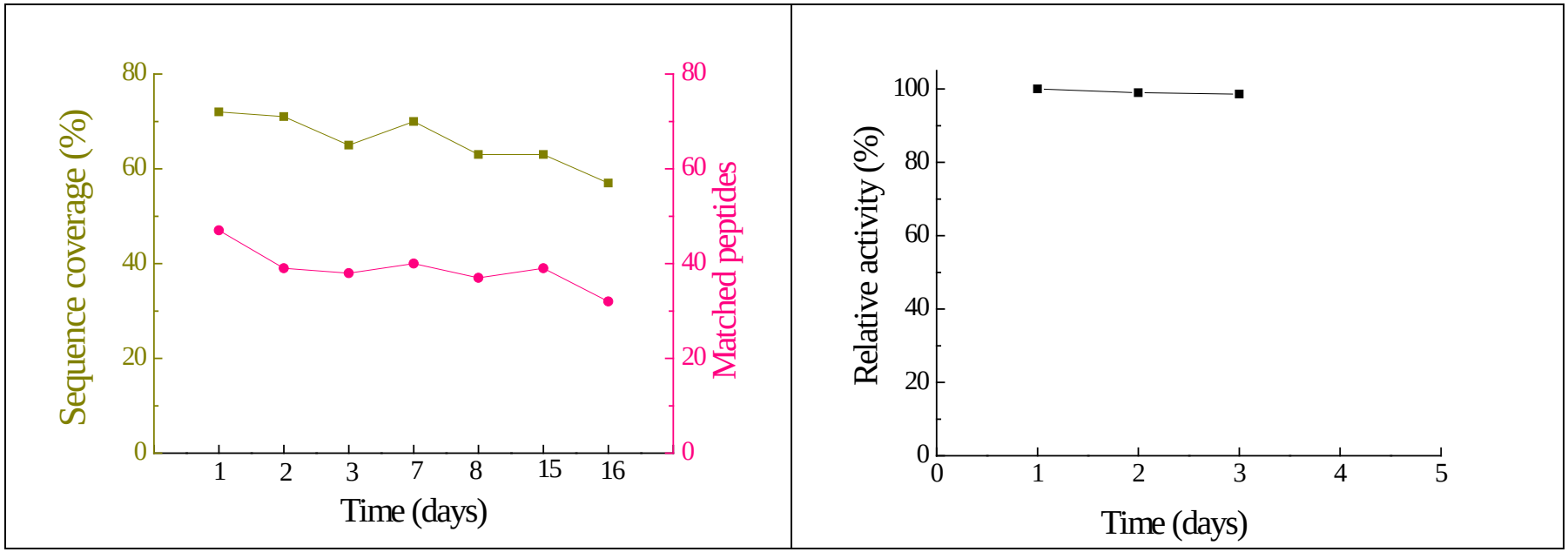


Figure S15 stability test of trypsin-FITC@CYCU-4. (left) BSA digestion efficiencies of trypsin-FITC@CYCU-4, (right) day-to-day activities of trypsin-FITC@CYCU-4 tested by BAPNA hydrolysis.

(The relative activity= (the absorbance value of immobilized reactor / the highest absorbance value of immobilized reactor*100%).)

16.

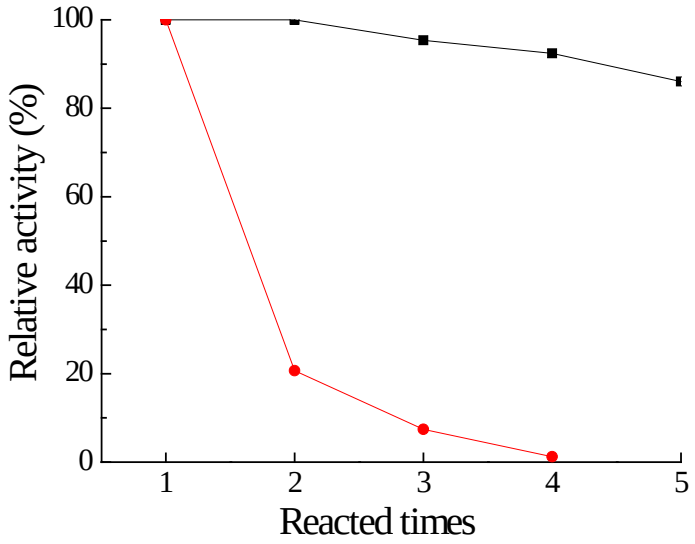


Figure S16 activity test of trypsin-FITC@CYCU-4 (■) and trypsin@CYCU-4 (●).

(The relative activity= (the absorbance value of immobilized reactor/ the highest absorbance value of immobilized reactor*100%)).

17.

Table S4 Literature survey of trypsin immobilized reactor for protein digestion

Materials	Time of surface modification	Time of trypsin immobilization	Sequence coverage/ matched peptides	Reference
Silica-coated magnetic nano particles (NPs)	Amino-functionalized: 3h	Trypsin coating: 27h	BSA: 50% Ovalbumin: 80% Myoglobin: 80% Carbonic anhydrase:80% Lactoglobulin: 50%	[6]
Magnetic Nanoparticles	Amino-functionalized: 6h Aldehyde-functionalized: 1.5 h Total :7.5 h	Trypsin covalently binding: 4 h	BSA: 38% Myoglobin: 80% Cytochrome c: 76 %	[7]
Mesoporous Silica (SBA-15)	N-(2-Aminoethyl)-3-aminopropyl-functionalized:18h	Trypsin adsorption: 10 mins	Myoglobin: 100%	[8]
Mesoporous silicate	Cyano-functionalized: 30 h	Trypsin adsorption: 16 h	Cytochrome c: 63 %	[9]
Polymer monolith	Aldehyde functionalization: 8h	Trypsin covalently binding: 30 h	BSA: 41%	[10]
Polymer monolith	Epoxy organic monoliths polymerization: few mins	Trypsin covalently binding: 26 h	Human serum albumin(HSA):78 % Bovine ribonuclease B (RNaseB):80% ß-Casein:49.76%	[11]
Polymer grafted magnetic beads	Graft copolymerization of methacrylic acid: 60h	Trypsin covalently binding: 3 h	Cytochrome c: 28 HSA: 20	[12]
Core/shell colloidal magnetic zeolite microspheres	Zeolite surface modification: 3 days	Trypsin adsorption: 1 h	BSA: 25% Myoglobin: 89% Cytochrome c: 77 %	[13]
MOFs	No necessity of modification	Trypsin adsorption: 30 mins	BSA: 72 %	[this work]

18.

Table S5. Comparison of the proteolytic efficiency of various trypsin-FITC@MOF bioreactor and free trypsin-FITC digestion (for 18h

incubation)

Position	MS	Peptides sequence	Free trypsin-FITC		MIL-53 (Al)		DUT-4 (Al)		DUT-5 (Al)		MIL-101 (Cr)		CYCU-4			"Al(III) + H_2SDC" material (non-porous).	
		Incubation time	18h	US 2 min	US 2 min		US 2 min		US 2 min		US 2 min		18h	US 2 min		US 2 min	
		Consecutive digestion	1st	1st	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	1st	2nd	1st	2nd
		Sequence coverage (%)	49	72	22	14	38	-	55	-	56	54	52	72	71	39	4
		Matched peptides	24	44	9	7	21	-	28	-	37	30	27	47	40	22	2
35-44	1248.625	R.FKDLGEEHFK.G		√									√	√	√		
45-65	2491.305	K.GLVLIAFSQYLQQCP FDEHVK.L		√										√			
45-75	3635.918	K.GLVLIAFSQYLQQCP FDEHVKLVNELTEFAK. T		√									√	√	√		
66-75	1162.645	K.LVNELTEFAK.T	√	√		√	√		√		√	√	√	√	√	√	
66-88	2607.308	K.LVNELTEFAKTCVAD ESHAGCEK.S							√		√		√	√	√	√	
76-100	2863.358	K.TCVADESHAGCEKSL HTLFGDELCK.V							√		√	√		√	√	√	
89-100	1418.725	K.SLHTLFGDELCK.V	√	√	√		√		√		√	√	√		√	√	
89-105	1944.848	K.SLHTLFGDELCKVAS LR.E					√				√	√		√	√	√	
101-117	2003.805	K.VASLRETYGDMADC		√								√					

		CEK.Q														
106-117	1477.525	R.ETYGDMADCCEK.Q											√			
123-138	1900.928	R.NECFLSHKDDSPDLP K.L								√			√	√		
139-151	1575.805	K.LKPDPNTLCDEFK.A		√			√		√	√		√	√			
139-155	2019.045	K.LKPDPNTLCDEFKAD EK.K	√	√			√				√					
161-167	926.683	K.YLYEIAR.R	√	√								√				
161-168	1082.645	K.YLYEIARR.H		√												
168-183	2044.058	R.RHPYFYAPELLYYAN K.Y			√							√				
184-197	1746.765	K.YNGVFQECCQAEDK. G								√	√		√	√		
184-204	2486.108	K.YNGVFQECCQAEDK GACLLPK.I	√	√			√				√	√		√		
198-204	757.493	K.GACLLPK.I								√	√		√	√		
198-209	1387.505	K.GACLLPKIETMR.E								√	√					
223-232	1194.625	R.CASIQKFGER.A		√		√	√								√	
233-241	1000.645	R.ALKAWSVAR.L		√	√	√										
236-241	688.393	K.AWSVAR.L														
246-256	1293.685	K.FPKAEFVEVTK.L								√	√		√			
249-256	921.523	K.AEFVEVTK.L		√						√			√	√		
249-263	1692.038	K.AEFVEVTKLVTDLTK. V		√			√			√		√	√	√	√	
257-263	788.503	K.LVTDLTK.V	√	√									√			

257-266	1152.505	K.LVTDLTKVHK.E						√				√		
267–280	1748.705	K.ECCHGDLLECADDR.A						√	√		√	√		
267-285	2246.945	K.ECCHGDLLECADDRADLAK.Y	√	√			√	√	√	√	√	√	√	√
286-297	1441.965	K.YICDNQDTISSK.L	√	√		√	√	√	√	√				√
286-299	1683.128	K.YICDNQDTISSKLK.E				√					√	√		
298-309	1531.805	K.LKECCDKPLLEK.S	√	√		√	√			√		√	√	√
319-340	2457.218	K.DAIPENLPPLTADFAEDKDVCK.N	√						√		√			
341-359	2300.085	K.NYQEAKDAFLGSFLYEYSR.R		√		√	√	√	√		√	√		
347-359	1566.765	K.DAFLGSFLYEYSR.R	√	√			√			√	√			
360-371	1438.825	R.RHPEYAVSVLLR.L		√		√	√				√			
361-371	1282.765	R.HPEYAVSVLLR.L		√		√				√				
372-386	1813.865	R.LAKEYEATLEECCAK.D		√						√				
375-386	1501.645	K.EYEATLEECCAK.D							√			√		
375-399	3037.238	K.EYEATLEECCAKDDPHACYSTVFDK.L						√			√			
387-399	1553.745	K.DDPHACYSTVFDK.L						√	√		√	√		
387-401	1794.865	K.DDPHACYSTVFDKLK.H		√			√	√	√			√		
402-412	1304.725	K.HLVDEPQNLIK.Q	√	√		√		√		√	√	√	√	√
402-420	2354.171	K.HLVDEPQNLIKQNCD		√			√			√	√	√		

		QFEK.L																
413-433	2528.211	K.QNCDQFEKLGEYGF QNALIVR.Y	√	√			√		√		√	√	√	√	√	√		
421-433	1478.845	K.LGEYGFQNALIVR.Y	√	√					√		√	√	√	√	√	√		
437-451	1638.968	R.KVPQVSTPTLVEVSR. S	√	√	√	√	√		√		√	√	√	√		√		√
438-451	1510.905	K.VPQVSTPTLVEVSR.S									√	√	√	√				
460-482	2871.398	R.CCTKPESERMPCTED YLSLILNR.L		√	√		√		√		√	√	√	√				
469-482	1723.868	R.MPCTEDYLSLILNR.L	√	√					√		√	√	√					
483-495	1538.845	R.LCVLHEKTPVSEK.V	√	√			√		√		√	√	√	√				
499-507	1137.585	K.CCTESLVNR.R		√					√		√	√		√	√			
499-523	2999.348	K.CCTESLVNRRPCFSAL TPDETYVPK.A	√		√	√	√		√				√	√	√	√		
508-523	1879.925	R.RPCFSALTPDETYVP K.A	√	√		√	√		√		√	√	√			√		
508-528	2470.371	R.RPCFSALTPDETYVP KAFDEK.L		√			√		√		√	√		√	√			
524–544	2497.238	K.AFDEKLFTFHADICT LPDTEK.Q		√	√		√		√		√	√	√	√	√			
529-544	1906.945	K.LFTFHADICTLPDTEK .Q	√	√		√	√		√		√	√				√		
529-547	2276.251	K.LFTFHADICTLPDTEK QIK.K		√	√				√		√	√		√	√	√		
548-557	1141.765	K.KQTALVELLK.H	√	√	√		√		√		√	√	√	√				

549-557	1013.665	K.QTALVELLK.H	√	√		√			√				√							
549-561	1503.968	K.QTALVELLKHKPK.A																		
562-580	2198.228	K.ATEEQLKTVMENFVA FVDK.C		√							√				√	√				
569-580	1398.745	K.TVMENFVAFVDK.C	√										√		√	√				
581-597	1926.818	K.CCAADDKEACFAVE GPK.L		√			√		√			√			√					
588-597	1106.585	K.EACFAVEGPK.L													√	√				
598-607	1000.665	K.LVVSTQTALA.-	√	√		√	√		√			√	√					√		√

19.

Table S6 Proteolytic efficiency of trypsin-FITC@CYCU-4 and trypsin-FITC@MIL-101(Cr) bioreactors for consecutive digestion

Position	MS	Amino acid sequences	Trypsin-FITC@CYCU-4[a]					Trypsin-FITC@MIL-101(Cr)[b]		
		Consecutive digestion	1st	2nd	3rd	4th	5th	1st	2nd	3rd
		Sequence coverage (%)	72	71	65	70	63	56	54	-[c]
		Matched peptides	47	39	38	40	37	37	30	-
35 – 44	1248.625	R.FKDLGEEHFK.G	√	√	√					
45 – 65	2491.305	K.GLVLIAFSQYLQQCPFDEHVK.L	√							
45 – 75	3635.918	K.GLVLIAFSQYLQQCPFDEHVKLVNELTEFAK.T	√	√	√					
66 – 75	1162.645	K.LVNELTEFAK.T	√	√	√	√	√	√	√	
66 – 88	2607.308	K.LVNELTEFAKTCVADESHAGCEK.S	√	√	√	√		√		
76 – 88	1462.765	K.TCVADESHAGCEK.S				√	√	√	√	
76 – 100	2863.358	K.TCVADESHAGCEKSLHTLFGDELCK.V	√	√	√	√	√	√	√	
89 – 100	1418.725	K.SLHTLFGDELCK.V		√		√	√	√	√	
89 – 105	1945.148	K.SLHTLFGDELCKVASLR.E	√	√			√		√	
101 - 117	2003.805	K.VASLRETYGDMADCCEK.Q								
106 - 117	1477.525	R.ETYGDMADCCEK.Q	√					√		
123 - 138	1900.928	R.NECFLSHKDDSPDLPK.L	√	√	√	√		√	√	
139 - 151	1575.805	K.LKPDPNTLCDEFK.A	√	√	√	√	√			
139 - 155	2019.045	K.LKPDPNTLCDEFKADEK.K								
161 - 167	926.683	K.YLYEIAR.R	√							
161 - 168	1082.645	K.YLYEIARR.H								
168 - 183	2044.058	R.RHPYFYAPELLYYANK.Y	√		√			√	√	
184 - 197	1746.765	K.YNGVFQECCQAEDK.G	√	√	√	√	√		√	

184 - 204	2486.108	K.YNGVFQECCQAEDKGACLLPK.I		√				√	√		
198 - 204	757.493	K.GACLLPK.I	√	√	√		√	√	√		
198- 209	1387.505	K.GACLLPKIETMR.E									
223 - 232	1194.625	R.CASIQKFGER.A			√	√	√				
233 - 241	1000.645	R.ALKAWSVAR.L				√	√				
236 - 241	688.393	K.AWSVAR.L						√	√		
246 - 256	1293.685	K.FPKAEFVEVTK.L	√					√			
249 - 256	921.523	K.AEFVEVTK.L	√	√	√			√		√	
249 - 263	1692.038	K.AEFVEVTKLVTDLTK.V	√	√	√	√	√				
257 - 263	788.503	K.LVTDLTK.V	√			√		√			
267 - 280	1748.705	K.ECCHGDLLECADDR.A	√	√	√	√		√	√		
267 - 285	2246.945	K.ECCHGDLLECADDRADLAK.Y	√	√	√			√	√		
286 - 297	1441.965	K.YICDNQDTISSK.L				√		√	√		
286 - 299	1683.128	K.YICDNQDTISSKLK.E	√	√	√	√	√				
298 - 309	1531.805	K.LKECCDKPLLEK.S		√		√	√				
319 - 340	2457.218	K.DAIPENLPPLTADFAEDKDVCK.N	√			√	√		√		
341 - 359	2300.085	K.NYQEAKDAFLGSFLYEYSR.R	√	√	√			√	√		
347 - 359	1566.765	K.DAFLGSFLYEYSR.R	√	√	√			√			
360 - 371	1438.825	R.RHPEYAVSVLLR.L		√			√	√			
361 - 371	1282.765	R.HPEYAVSVLLR.L	√		√	√	√				
372 - 386	1813.865	R.LAKEYEATLEECCAK.D	√			√					
375 - 386	1501.645	K.EYEATLEECCAK.D		√		√			√		
375 - 399	3037.238	K.EYEATLEECCAKDDPHACYSTVFDK.L	√					√			
387 - 399	1553.745	K.DDPHACYSTVFDK.L	√	√	√			√	√		

387 - 401	1794.865	K.DDPHACYSTVFDKLK.H		√	√			√	√		
402 - 412	1304.725	K.HLVDEPQNLIK.Q	√	√	√		√	√			
402 - 420	2354.171	K.HLVDEPQNLIKQNCDQFEK.L	√	√	√	√					√
413 - 433	2528.211	K.QNCDQFEKLGEYGFQNALIVR.Y	√	√	√	√	√	√	√		
421 - 433	1478.845	K.LGEYGFQNALIVR.Y	√	√	√	√	√	√	√		
437 - 451	1638.968	R.KVPQVSTPTLVEVSR.S	√		√	√		√	√		
438 - 451	1510.905	K.VPQVSTPTLVEVSR.S	√	√	√	√	√				
460 - 482	2871.398	R.CCTKPESERMPCTEDYLSLILNR.L	√	√	√	√	√	√			√
469 - 482	1723.868	R.MPCTEDYLSLILNR.L	√	√	√	√	√	√			
483 - 495	1538.845	R.LCVLHEKTPVSEK.V	√					√	√		
499 - 507	1137.585	K.CCTESLVNR.R	√	√	√	√		√	√		
499 - 523	2999.348	K.CCTESLVNRRPCFSALTPDETYVPK.A	√	√	√	√					
508 - 523	1879.925	R.RPCFSALTPDETYVPK.A				√	√	√	√		
508 - 528	2470.371	R.RPCFSALTPDETYVPKAFDEK.L	√	√	√	√	√	√	√		
524 - 544	2497.238	K.AFDEKLFTFHADICTLPDTEK.Q	√	√	√			√			
529 - 544	1906.945	K.LFTFHADICTLPDTEK.Q			√	√	√	√	√		
529 - 547	2276.251	K.LFTFHADICTLPDTEKQIK.K	√	√	√	√	√	√	√		
548 - 557	1141.765	K.KQTALVELLK.H	√	√	√	√					
549 - 557	1013.665	K.QTALVELLK.H				√	√				
549 - 561	1503.968	K.QTALVELLKHKPK.A				√	√				
562 - 580	2198.228	K.ATEEQLKTVMENFVAFVDK.C	√	√				√			
569 - 580	1398.745	K.TVMENFVAFVDK.C	√	√	√						
581 - 597	1926.818	K.CCAADDKEACFAVEGPK.L	√						√		
588 - 597	1106.585	K.EACFAVEGPK.L	√	√	√	√					

598 - 607	1000.665	K.LVVSTQTALA.-	√

[a] 1st batch synthesized CYCU-4 used as trypsin-immobilized MOF reactor.

[b] 1st batch synthesized MIL-101(Cr) used as trypsin-immobilized MOF reactor

[c] No BSA digests were measured.

20.

Table S7 Proteolytic efficiency of trypsin-FITC@CYCU-4 and trypsin-FITC@MIL-101(Cr) bioreactors prepared from different batches

Position	MS	Amino acid sequences	Trypsin-FITC@CYCU-4			Trypsin-FITC@MIL-101(Cr)		
		Batch	1	2	3	1	2	3
		Sequence coverage (%)	72	63	64	56	45	46
		Matched peptides	47	33	38	37	25	25
35 - 44	1248.625	R.FKDLGEEHFK.G	√					
45 - 65	2491.305	K.GLVLIAFSQYLQQCPFDEHVK.L	√	√				
45 - 75	3635.918	K.GLVLIAFSQYLQQCPFDEHVKLVNELTEFAK.T	√	√				
66 - 75	1162.645	K.LVNELTEFAK.T	√	√	√	√	√	√
66 - 88	2607.308	K.LVNELTEFAKTCVADESHAGCEK.S	√		√	√		√
76 - 100	2863.358	K.TCVADESHAGCEKSLHTLFGDELCK.V	√	√	√	√	√	√
89 - 100	1418.725	K.SLHTLFGDELCK.V		√	√	√	√	√
89 - 105	1945.148	K.SLHTLFGDELCKVASLR.E	√	√	√	√	√	√
101 - 117	2003.805	K.VASLRETYGDMADCCEK.Q						
106 - 117	1477.525	R.ETYGDMADCCEK.Q	√		√			
123 - 138	1900.928	R.NECFLSHKDDSPDLPK.L	√	√	√	√		
139 - 151	1575.805	K.LKPDPNTLCDEFK.A	√		√	√		√
139 - 155	2019.045	K.LKPDPNTLCDEFKADEK.K			√		√	
161 - 167	926.683	K.YLYEIAR.R	√					
161 - 168	1082.645	K.YLYEIARR.H						
168 - 183	2044.058	R.RHPYFYAPELLYYANK.Y	√	√	√			
184 - 197	1746.765	K.YNGVFQECCQAEDK.G	√		√	√		√
184 - 204	2486.108	K.YNGVFQECCQAEDKGACLLPK.I			√		√	
198 - 204	757.493	K.GACLLPK.I	√			√		√

223 - 232	1194.625	R.CASIQKFGER.A				√	√	√
233 - 241	1000.645	R.ALKAWSVAR.L					√	
236 - 241	688.393	K.AWSVAR.L		√	√			
246 - 256	1293.685	K.FPKAEFVEVTK.L	√					
249 - 256	921.523	K.AEFVEVTK.L	√			√		√
249 - 263	1692.038	K.AEFVEVTKLVTDLTK.V	√	√	√	√	√	
257 - 263	788.503	K.LVTDLTK.V	√	√	√	√		
267 - 280	1748.705	K.ECCHGDLLECADDR.A	√		√			
267 - 285	2246.945	K.ECCHGDLLECADDRADLAK.Y	√	√	√	√		
286 - 297	1441.965	K.YICDNQDTISSK.L				√	√	√
286 - 299	1683.128	K.YICDNQDTISSKLK.E	√	√		√		
298 - 309	1531.805	K.LKECCDKPLLEK.S		√	√	√	√	√
319 - 340	2457.218	K.DAIPENLPPLTADFAEDKDVCK.N	√	√	√			
341 - 359	2300.085	K.NYQEAKDAFLGSFLYEYSR.R	√	√				
347 - 359	1566.765	K.DAFLGSFLYEYSR.R	√		√			
360 - 371	1438.825	R.RHPEYAVSVLLR.L				√	√	√
361 - 371	1282.765	R.HPEYAVSVLLR.L	√	√		√		
372 - 386	1813.865	R.LAKEYEATLEECCAK.D	√	√		√		
375 - 386	1501.645	K.EYEATLEECCAK.D		√	√			
375 - 399	3037.238	K.EYEATLEECCAKDDPHACYSTVFDK.L	√	√	√			
387 - 399	1553.745	K.DDPHACYSTVFDK.L	√	√	√			
387 - 401	1794.865	K.DDPHACYSTVFDKLK.H		√		√		√
402 - 412	1304.725	K.HLVDEPQNLIK.Q	√	√	√	√	√	√
402 - 420	2354.171	K.HLVDEPQNLIKQNCDQFEK.L	√		√	√	√	√
413 - 433	2528.211	K.QNCDQFEKLGEYGFQNALIVR.Y	√	√	√	√	√	√

421 - 433	1478.845	K.LGEYGFQNALIVR.Y	√						√		√
437 - 451	1638.968	R.KVPQVSTPTLVEVSR.S	√		√		√		√		√
438 - 451	1510.905	K.VPQVSTPTLVEVSR.S	√				√		√		
460 - 482	2871.398	R.CCTKPESERMPCTEDYLSLILNR.L	√		√		√		√		√
469 - 482	1723.868	R.MPCTEDYLSLILNR.L	√		√		√				
483 - 495	1538.845	R.LCVLHEKTPVSEK.V	√						√		
499 - 507	1137.585	K.CCTESLVNR.R	√		√		√		√		
499 - 523	2999.348	K.CCTESLVNRRPCFSALTPDETYVPK.A	√		√		√		√		√
508 - 523	1879.925	R.RPCFSALTPDETYVPK.A							√		√
508 - 528	2470.371	R.RPCFSALTPDETYVPKAFDEK.L	√		√		√				
524 - 544	2497.238	K.AFDEKLFTFHADICTLPDTEK.Q	√		√		√		√		√
529 - 544	1906.945	K.LFTFHADICTLPDTEK.Q							√		√
529 - 547	2276.251	K.LFTFHADICTLPDTEKQIK.K	√						√		√
548 - 557	1141.765	K.KQTALVELLK.H	√		√		√		√		√
549 - 557	1013.665	K.QTALVELLK.H					√		√		√
549 - 561	1503.968	K.QTALVELLKHKPK.A					√				
562 - 580	2198.228	K.ATEEQLKTVMENFVAFVDK.C	√		√		√				
569 - 580	1398.745	K.TVMENFVAFVDK.C	√								
581 - 597	1926.818	K.CCAADDKEACFAVEGPK.L	√						√		
588 - 597	1106.585	K.EACFAVEGPK.L	√								
598 - 607	1000.665	K.LVVSTQTALA.-									

21.

Table S8 Comparison of the proteolytic efficiency of trypsin-FITC@CYCU-4 bioreactors prepared with different FITC concentrations*

Position	MS	Amino acid sequences	Trypsin-FITC@CYCU-4

		FITC concentration (mM)	1	2.5	5	7.5	15
		Sequence coverage (%)	32	45	72	68	36
		Matched peptides	15	28	47	43	19
35 - 44	1248.625	R.FKDLGEEHFK.G			√		
45 - 65	2491.305	K.GLVLIAFSQYLQQCPFDEHVK.L			√		
45 - 75	3635.918	K.GLVLIAFSQYLQQCPFDEHVKLVNELTEFAK.T			√		
66 - 75	1162.645	K.LVNELTEFAK.T	√	√	√	√	√
66 - 88	2607.308	K.LVNELTEFAKTCVADESHAGCEK.S			√	√	
76 - 100	2863.358	K.TCVADESHAGCEKSLHTLFGDELCK.V			√	√	
89 - 100	1418.725	K.SLHTLFGDELCK.V		√		√	√
89 - 105	1945.148	K.SLHTLFGDELCKVASLR.E	√	√	√	√	√
101 - 117	2003.805	K.VASLRETYGDMADCCEK.Q		√		√	
106 - 117	1477.525	R.ETYGDMADCCEK.Q			√	√	
123 - 138	1900.928	R.NECFLSHKDDSPDLPK.L			√		
139 - 151	1575.805	K.LKPDPNTLCDEFK.A			√	√	
139 - 155	2019.045	K.LKPDPNTLCDEFKADEK.K				√	
161 - 167	926.683	K.YLYEIAR.R			√		
161 - 168	1082.645	K.YLYEIARR.H				√	
168 - 183	2044.058	R.RHPYFYAPELLYYANK.Y			√		
184 - 197	1746.765	K.YNGVFQECCQAEDK.G			√		
184 - 204	2486.108	K.YNGVFQECCQAEDKGACLLPK.I				√	
198 - 204	757.493	K.GACLLPK.I			√	√	√
223 - 232	1194.625	R.CASIQKFGER.A				√	√
233 - 241	1000.645	R.ALKAWSVAR.L					
236 - 241	688.393	K.AWSVAR.L					

246 - 256	1293.685	K.FPKAEFVEVTK.L			√		
249 - 256	921.523	K.AEFVEVTK.L	√	√	√	√	
249 - 263	1692.038	K.AEFVEVTKLVTDLTK.V			√	√	
257 - 263	788.503	K.LVTDLTK.V			√		
267 - 280	1748.705	K.ECCHGDLLECADDR.A			√	√	
267 - 285	2246.945	K.ECCHGDLLECADDRADLAK.Y	√	√	√	√	√
286 - 297	1441.965	K.YICDNQDTISSK.L				√	
286 - 299	1683.128	K.YICDNQDTISSKLK.E		√	√	√	√
298 - 309	1531.805	K.LKECCDKPLLEK.S					
319 - 340	2457.218	K.DAIPENLPPLTADFAEDKDVCK.N		√	√	√	
341 - 359	2300.085	K.NYQEAKDAFLGSFLYEYSR.R	√	√	√	√	
347 - 359	1566.765	K.DAFLGSFLYEYSR.R		√	√	√	√
360 - 371	1438.825	R.RHPEYAVSVLLR.L		√		√	
361 - 371	1282.765	R.HPEYAVSVLLR.L			√	√	
372 - 386	1813.865	R.LAKEYEATLEECCAK.D			√		
375 - 386	1501.645	K.EYEATLEECCAK.D				√	
375 - 399	3037.238	K.EYEATLEECCAKDDPHACYSTVFDK.L		√	√		
387 - 399	1553.745	K.DDPHACYSTVFDK.L		√	√	√	√
387 - 401	1794.865	K.DDPHACYSTVFDKLK.H	√	√		√	
402 - 412	1304.725	K.HLVDEPQNLIK.Q	√	√	√	√	√
402 - 420	2354.171	K.HLVDEPQNLIKQNCDQFEK.L	√	√	√	√	√
413 - 433	2528.211	K.QNCDQFEKLGEYGFQNALIVR.Y	√	√	√	√	√
421 - 433	1478.845	K.LGEYGFQNALIVR.Y			√		√
437 - 451	1638.968	R.KVPQVSTPTLVEVSR.S	√	√	√	√	√
438 - 451	1510.905	K.VPQVSTPTLVEVSR.S			√		

460 - 482	2871.398	R.CCTKPESERMPCTEDYLSLILNR.L			√	√	
469 - 482	1723.868	R.MPCTEDYLSLILNR.L			√	√	
483 - 495	1538.845	R.LCVLHEKTPVSEK.V	√	√	√	√	√
499 - 507	1137.585	K.CCTESLVNR.R		√	√	√	
499 - 523	2999.348	K.CCTESLVNRRPCFSALTPDETYVPK.A		√	√		
508 - 523	1879.925	R.RPCFSALTPDETYVPK.A	√	√		√	
508 - 528	2470.371	R.RPCFSALTPDETYVPKAFDEK.L	√	√	√	√	√
524 - 544	2497.238	K.AFDEKLFTFHADICTLPDTEK.Q		√	√	√	√
529 - 544	1906.945	K.LFTFHADICTLPDTEK.Q		√		√	√
529 - 547	2276.251	K.LFTFHADICTLPDTEKQIK.K		√	√	√	
548 - 557	1141.765	K.KQTALVELLK.H			√		
549 - 557	1013.665	K.QTALVELLK.H					
549 - 561	1503.968	K.QTALVELLKHKPK.A	√	√		√	
562 - 580	2198.228	K.ATEEQLKTVMENFVAFVDK.C			√	√	
569 - 580	1398.745	K.TVMENFVAFVDK.C			√		
581 - 597	1926.818	K.CCAADDKEACFAVEGPK.L	√	√	√	√	√
588 - 597	1106.585	K.EACFAVEGPK.L			√		
598 - 607	1000.665	K.LVVSTQTALA.-					

* Various FITC concentrations ranging from 1 to 15 mM was add to react with trypsin protein at 2000 μg mL^{-1}.

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