Electronic Supplementary Material (ESI) for Nanoscale.

Co-immobilization of bi-enzymatic cascade into hierarchically porous MIL-53 for efficient 6'- sialyllactose production

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Materials and methods

Materials

Zirconium chloride ($ZrCl_4$), biphenyl-4,4-dicarboxylic acid (BPDC) and cresol red were purchased by Aladdin (Shanghai, China). CMP-Neu5Ac was purchased by Glycogene (Wuhan, China).

Synthesis of HP-UiO-67

HP-UiO-67 was synthesized according to the previous literature with some modifications[1]. Typically, 160 mg $ZrCl_4$ and 4.8 g dodecanoic acid were first dissolved in 40 ml DMF, then 82 mg BPDC was added. The solution was sealed in a 100 ml Teflon-lined stainless-steel vessel under autogenous pressure for 2 d at 120 °C. After the reaction, the precipitate was isolated by centrifugation and washed with 20 ml DMF for 3 times. To activate the sample, the powder was added to the mixture solution of 80 ml DMF and 0.4 ml hydrochloric acid for 12 hr at 90 °C. Finally, the powder was centrifuged and washed with 20 ml DMF for 3 times and 20 ml methanol for 3 times, and dried in a vacuum oven at 120 °C overnight.

Preparation of NmCSS&Pd26ST@HP-UiO-67

Typically, for the preparation of NmCSS&Pd26ST@HP-UiO-67, 0.16 mg NmCSS (6 nmol), 0.98 mg Pd26ST (18 nmol) and 10 mg HP-UiO-67 were stirred at 4 °C for 2 hr. Afterwards, NmCSS&Pd26ST@HP-UiO-67 was obtained by centrifugation and washed 3 times with ddH_2O.

Kinetic parameters assays

The kinetic parameters of NmCSS were measured according to pervious research with some modifications[2]. For CTP, experiments were carried out in 3 mM Tris-HCl buffer (pH = 8.5) containing 0.1 μM NmCSS, 0.13 mM cresol red, 5 mM Neu5Ac and 10 mM $MgCl_2$ at 37 °C. CTP concentration was varied from 0.005 mM to 0.15 mM. For Neu5Ac, experiments were carried out in 3 mM Tris-HCl buffer (pH = 8.5) containing 0.1 μM NmCSS, 0.13 mM cresol red, 5 mM CTP and 10 mM $MgCl_2$ at 37 °C. Neu5Ac concentration was varied from 0.1 mM to 2.5 mM.

The kinetic parameters of Pd26ST were measured according to pervious research with some modifications[3]. For CMP-Neu5Ac, experiments were carried out in 5 mM Tris-HCl buffer (pH = 8.5) containing 0.2 μM Pd26ST, 0.26 mM cresol red and 20 mM

lactose at 37 °C. CMP-Neu5Ac concentration was varied from 0.5 mM to 5 mM. For lactose, experiments were carried out in 5 mM Tris-HCl buffer (pH = 8.5) containing 0.2 μM Pd26ST, 0.26 mM cresol red and 10 mM CMP-Neu5Ac at 37 °C. Lactose concentration was varied from 1 mM to 20 mM.

For immobilized enzyme, free enzyme was replaced with enzyme-MOF containing the same amount of enzyme. The absorbance change was measured at a wavelength of 574 nm. The colorimetric assays were carried out using a microplate reader (Tecan, Infinite M200 pro). The data were collected every 10 s for 5 minutes. The kinetic parameters were obtained by nonlinear regression analysis of the Michaelis–Menten equation using Origin 2018.

Supplementary Figures

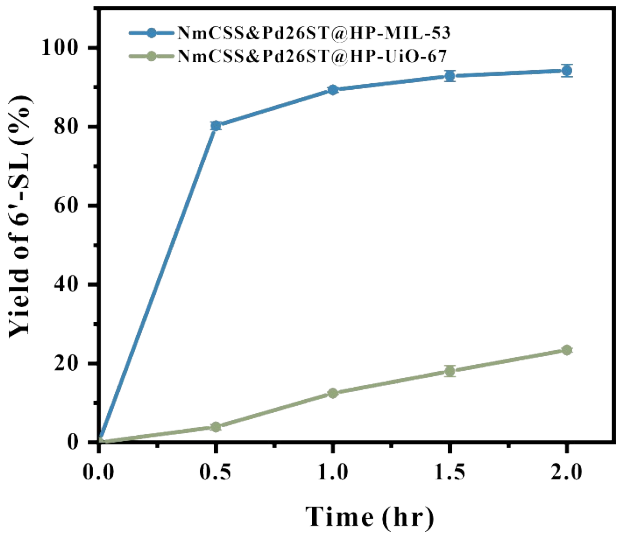

Fig. S1 Comparison of catalytic performance of NmCSS&Pd26ST@HP-MIL-53 and NmCSS&Pd26ST@HP-UiO-67.

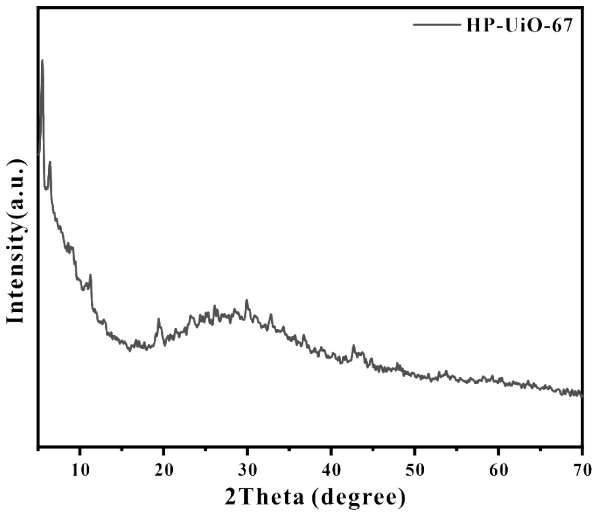

Fig. S2 XRD spectrum of HP-UiO-67

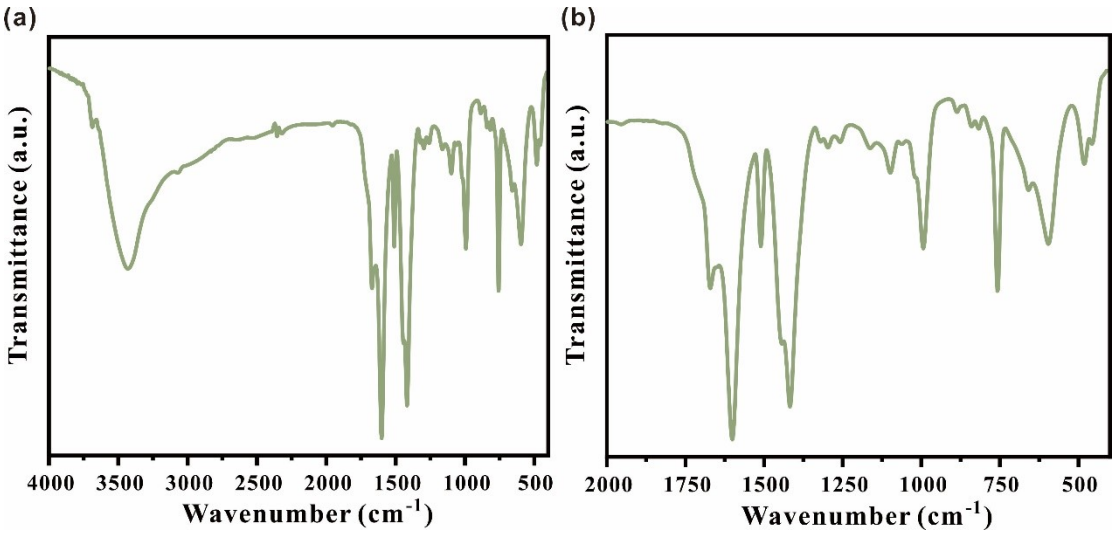

Fig. S3 FTIR spectra of HP-MIL-53 in different wavenumber ranges.

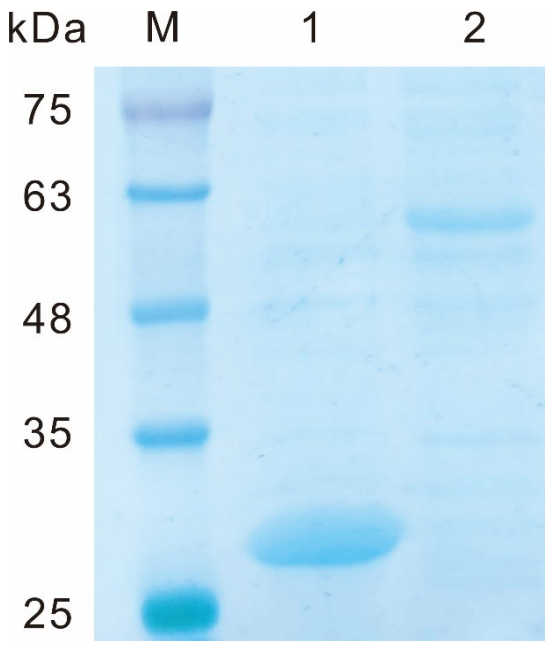

Fig. S4 SDS-PAGE analysis of purified proteins. Lanes: M, molecular weight marker; 1, NmCSS; 2, Pd26ST.

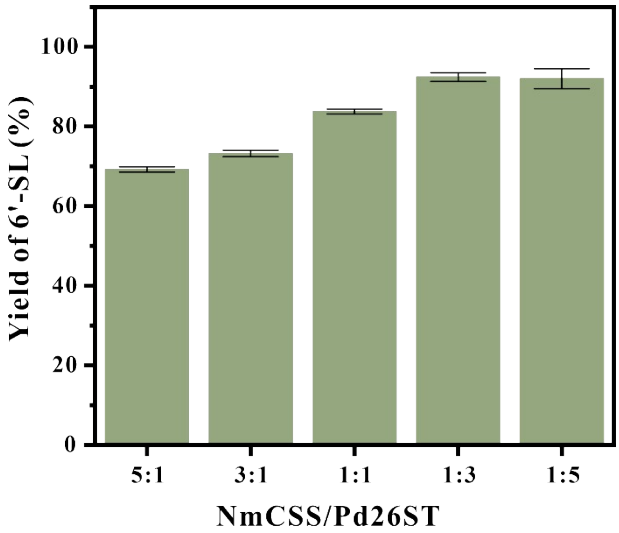

Fig. S5 Comparison of catalytic performance of free NmCSS and Pd26ST with different molar ratio of NmCSS to Pd26ST

Supplementary Table

Table S1 Comparison of enzyme immobilization capacities of different MOFs

MOFs	Enzyme	Capacity for enzyme (mg· g^{-1})	Ref.
HP-PCN-224(Fe)	glucose oxidase	192.6	[4]
ZIF-67	lipase	26.9	[5]
ZIF-90	lipase	132.0	[6]
ZIF-8	cellulase	176.2	[7]
Zn-MOF	peroxidase	109.9	[8]
Fe-BTC	laccase	45.2	[9]
UiO-66-NH_2	soybean epoxide hydrolase	87.3	[10]
Fe_3O_4@Cu-BTC	xylanase	80.7	[11]
HP-MIL-53	NmCSS and Pd26ST	226	this work

Table S2 Pore characteristics of HP-MIL-53 prepared with different amounts of dodecanoic acid

MOF	S_{BET} ($m^2 \cdot g^{-1}$)	V_{total} ($cm^3 \cdot g^{-1}$)	Average pore diameter (nm)
HP-MIL-53(10)	822.46	0.91	4.42
HP-MIL-53(20)	737.87	1.14	6.18
HP-MIL-53(30)	933.11	0.95	4.06

Table S3 Michaelis–Menten kinetic parameters of free and immobilized NmCSS and Pd26ST

Enzyme	Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}\cdot s^{-1}$)
NmCSS	CTP	0.014±0.002	5.26±0.17	375.71
	Neu5Ac	0.43±0.07	5.61±0.17	13.05
NmCSS@HP-MIL-53(20)	CTP	0.010±0.001	4.61±0.06	442.78
	Neu5Ac	0.40±0.03	4.94±0.10	12.35
Pd26ST	Lactose	6.32±1.66	7.60±0.63	1.20
	CMP-Neu5Ac	2.23±0.24	9.85±0.49	4.42
Pd26ST@HP-MIL-53(20)	Lactose	5.84±0.40	6.65±0.17	1.13
	CMP-Neu5Ac	1.90±0.19	8.40±0.43	4.42

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