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Supplementary information

Encapsulating and stabilizing enzymes using hydrogen-bonded organic frameworks

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Encapsulating and stabilizing enzymes using hydrogen-bonded organic frameworks

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Supplementary Method 1 | ammoniation of enzyme

1. Add 2.4 mg ethylenediamine (EDA) into 10 mL of deionized water in a plastic tube
2. Transfer 2 mL of prepared EDA solution (4.01 mM) in a 20 mL glass vial
3. Adjust the pH of the EDA solution to 4.5 using 1 M HCl.
4. Add 20 mg enzyme into the EDA solution, followed by adding 7.2 mg of N-(3-dimethylamino-propyl)-N'-ethylcarbodiimide hydrochloride (0.038 mmol).
5. Stir the mixed solution on ice for 120 min to enable the ammoniation.
6. Transfer the ammoniated enzyme solution to a dialysis bag (molecular weight cut-off MWCO= 8 kDa), and remove the excess reagents and salts by dialysis in deionized water for 18 h (renew the deionized water every 6 h).
7. Concentrate the ammoniated enzyme to 2 mg/mL using deionized water.

■ **PAUSE POINT** This ammoniated enzyme solution can be stored at 4 °C in the refrigerator overnight if desired.

Supplementary Table 1. Summary of the protein loading efficiencies using this method and the isoelectric point of proteins

proteins	Loading efficiency[a] (%, w/w)	isoelectric point[b]
BSA	33.2	5.8
Cyt c	32.7	9.1
HRP	34.2	9.6
CAT	47.9	7.6
MB	44.6	8.7
Pepsin	24.3	3.7
GOx	28.0	5.0
OVA	30.0	5.2
TRF	36.9	8.4

[a]the loading efficiency is obtained from our primary research paper (Ref. 1)

[b]this parameters were supported by the SIB Bioinformatics Resource Portal, ExPASy. (https://www.expasy.org/)

Supplementary Table 2[a]. Comparison of the loading efficiencies between this protocol and other in situ MOF, COF and HOF methods for protein encapsulation

carriers	proteins	Loading (w/w, %)	Reference
ZIF-8	GOx, HRP, β-galactosidase, alcohol dehydrogenase, lactate dehydrogenase	< 5	*Nat. Catal.* 1, 689– 695 (2018).
ZIF-8	HRP, Cyt c, GOx	0.25-4.2	*Angew. Chem. Int. Ed.* 59, 13947-13954 (2020).
ZIF-8	GOx, HRP	8.45%, 4.71	*Sci. Adv.* 6, eaax5785 (2020).
ZIF-8	Cyt c, HRP, CAT, Urate oxidase, alcohol dehydrogenase	0.7-4.2	*Angew. Chem. Int. Ed.* 59, 2867 – 2874 (2020).
ZIF-8	GOx	~10	*Nat. Commun.* 10, 5165 (2019).
ZIF-90	CAT	6.0	*J. Am. Chem. Soc.* 139, 6530−6533 (2017).
ZIF-90	CAT	1.3	*J. Am. Chem. Soc.* 141, 2348−2355 (2019)
MAF-7[b]	CAT	3.8, 7.0	*J. Am. Chem. Soc.* 141, 2348−2355 (2019)

MAF-2[c]	GOx	7.0	*Chem. Eur. J.* 25,5463 – 5471 (2019)
UIO-66-NH_2[d]	β-glucosidase, invertase, β-galactosidase	~13.5, ~14.8, 12.3	*Nat. Commun.* 10, 5002 (2019).
COFs TpPa-1[e]	Cyt c, HRP, lipase	21.7, 27.8, 12.5,	*Cell Rep. Phys. Sci.* 2022, 3, 101153
NKCOF-98[f], NKCOF-99[g],	Lipase, Cyt c, GOx, BSA	~20.6-~42.5	*Angew. Chem.Int. Ed.* 2022, 61, e202208744
BioHOF-1[h]	CAT, alcohol oxidase	~6.0, ~2.9	*J. Am. Chem. Soc.* 2019, 141, 36, 14298–14305
HOF-101	BSA, Cyt c, HRP, CAT, MB, Pepsin, GOx, OVA, TRF	24.3-47.9	This method

[a]Supplementary Table 2 is adapted from our primary research paper (Ref. 1)

[b]MAF-7: Metal azolate frameworks-7, a Zn-MOFs with 3‐methyl‐1,2,4‐triazole as linker

[c]MAF-2: Metal azolate frameworks-2, a Cu-MOFs with 3,5‐diethyl‐1,2,4‐triazole as linker

[d]UIO-66: Zr−MOF with 1,4-benzene-dicarboxylate as linker

[e]COF TpaPa-1, a COF constructed by 1,3,5-triformylphloroglucinol and *p*-phenylenediamine

[f]NKCOF-98, a COF constructed by 5-bis(2-methoxyethoxy)terephthalohydrazide and 1,3,5-triformylbenzene

[g]NKCOF-99, a COF constructed by hydrazinehydrate and 1,3,5-triformylbenzene

[h]BioHOF-1, a HOF constructed by *tetrakis*(4-amidiniumphenyl)methane and *tetrakis*(4-carboxylphenyl)methane

Supplementary Reference

1. Chen, G. et al. Protein-directed, hydrogen-bonded biohybrid framework. *Chem*, **7**, 2722–2742 (2021).