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Supplementary Information for Cyclodextrin metal-organic framework-based protein biocomposites

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Materials

Chemical reagents for γ-CD-MOF and β-CD-MOF syntheses were obtained from several suppliers and used as received unless specifically indicated. γ-Cyclodextrin was bought from Fischer Scientific. β-Cyclodextrin, Potassium acetate, Bovine Serum Albumin, Catalase, Myoglobin from Sigma Aldrich. Interleukin-2 from Med Chem Express. Milli-Q water (ρ > 18 MΩ cm) was used as the solvent for all aqueous solutions.

Synthesis of γ-CD-MOFs

γ-CD-MOFs were synthesized by mixing γ-Cyclodextrin (γ-CD) (283 mg, 0.22 mmol) with potassium acetate (170 mg 1.73 mmol) in 2 ml of Milli-Q water. Samples were then heated for 1 minute in an oven at 80 degrees and vortexed for 20 seconds or until complete dissolution was reached. The solutions were cooled down in a water bath at room temperature, put in a 15 ml centrifuge tube. 6 ml of methanol were added rapidly from a glass cylinder, and vortexed for 3 seconds. The solution was centrifuged at 10000 rpm for 10 minutes. The precipitate was separated from the solvent, washed with methanol, and centrifuged. For the drying process, the crystals were dried inside the centrifuge tube under airflow for 1-hour without using the vacuum. Synthesis resulted in a pellet of ~1 cm in diameter.

Synthesis of protein @ γ-CD-MOFs

Protein @ γ-CDMOFs were synthesized by mixing γ-Cyclodextrin (γ-CD) (283 mg, 0.22 mmol) with potassium acetate (170 mg 1.73 mmol) in 2 ml of Milli-Q water. Samples were then heated for 1 minute in an oven at 80 degrees and vortexed for 20 seconds or until complete dissolution was reached. The solutions were cooled down in a water bath at room temperature, put in a 15 ml centrifuge tube. 4 mg of Bovine Serum Albumin was dissolved in 200 µL Milli-Q water and added to the 15ml centrifuge tube. 6 ml of methanol were added rapidly from a glass cylinder, and vortexed for 3 seconds. The precipitate was then transferred to 2 ml centrifuge tube; the solution was then centrifuged at 10,000 rpm for 10 minutes; The precipitate was separated from the solvent, washed with fresh methanol, and centrifuged again under the same conditions. The crystals were dried inside the centrifuge tube under airflow for 1-hour, forming a pellet of 1 cm in diameter. The same synthesis was also repeated for Catalase and Myoglobin.

Synthesis of Interleukin-2 @ γ-CD-MOF

A solution of 2 ml of 283 mg of γ-cyclodextrin and 170 mg of potassium acetate was prepared. The solution was heated for 1 minute in an oven at 80 degrees and then vortexed for 20 seconds or until complete dissolution was reached. 100 µL of solution was injected into the vial containing the 50µg of IL-2 as received. Once the total dissolution of the IL-2 was confirmed, 200 µL MeOH was added quickly, and the solution vortexed for 2 seconds. After 10 minutes, the solution was centrifuged, the supernatant separated from the solid, and the solid dried with airflow for one hour.

Synthesis of β-CD-MOFs

To synthesize β-CD-MOFs, β-cyclodextrin (β-CD) (100mg, 0.089 mmol) and potassium acetate 60 mg (0.611 mmol) were added to 2 ml of Milli-Q water. The solution was left in the oven at 80 degrees until complete dissolution. The solution was cooled down in a bath at room temperature and transferred to a 15ml centrifuge tube. 6ml of acetonitrile was added, the solution was let to rest for several minutes and then vortexed. The solution was then centrifuged at 10,000 rpm for 10 minutes to obtain a precipitate. The precipitate was washed with fresh acetonitrile and centrifuged again under the same conditions. The crystals were dried inside the centrifuge tube under airflow for 1-hour, giving a pellet of 1 cm of diameter.

Synthesis of protein @ β-CD-MOFs

β-CD (100mg, 0.089 mmol) was added with potassium acetate (60 mg, 0.611 mmol) in 2 ml of Milli-Q water. The solution was left in the oven at 80 degrees until complete dissolution. The solution was cooled down in a bath at room temperature and transferred to a 15ml centrifuge tube. 4 mg of Bovine Serum Albumin was dissolved in 200 µL of Milli-Q water and added to the be β-CD/acetate mixture. After one hour, 6 ml of acetonitrile was added, let it rest for 5 minutes, and vortexed for 3 seconds following the addition. The solution was centrifuged at 10,000 rpm for 10 minutes to obtain a precipitate. The precipitate was washed with fresh acetonitrile and centrifuged again under the same conditions. The crystals were dried inside the centrifuge tube under airflow for 1-hour, giving a pellet of 1 cm of diameter. The same synthesis was also repeated for 4 mg Catalase and 4 mg Myoglobin.

Synthesis of Interleukin-2 @ β-CD-MOFs

A 2 ml solution containing 100 mg of *β*-CD and 60 mg of potassium acetate was prepared. 100 µL of the prepared solution was injected into a vial containing 50µg of IL-2 as received. Once dissolution of IL-2 was achieved, 300 µL acetonitrile was added to the solution, let it rest for 5 minutes, and then vortexed for 2 seconds. After 10 minutes, the solution was centrifuged to obtain a precipitate. The precipitate was washed with fresh acetonitrile and dried under airflow for 1- hour.

Synthesis of BSA @ γ- / β-CD-MOF

β-CD (100mg, 0.089 mmol) and γ-CD (110mg, 0.089 mmol) were added with potassium acetate (134 mg, 1.36 mmol) in 2 ml of Milli-Q water. The solution was left in the oven at 80 degrees until complete dissolution. The solution was cooled down in a bath at room temperature and transferred to a 15ml centrifuge tube. 4 mg of Bovine Serum Albumin was dissolved in 200 µL of Milli-Q water and added to the be *γ- / β*-CD/acetate mixture. 6 ml of acetonitrile was added, and the solution was let to rest for several minutes and then vortexed. The solution was then centrifuged at 10,000 rpm for 10 minutes to obtain a precipitate. The precipitate was washed with fresh acetonitrile and centrifuged again under the same conditions. The crystals were dried inside the centrifuge tube under airflow for 1-hour.

Encapsulation Efficiency

Encapsulation Efficiency was determined for BSA, Catalase, Myoglobin, and Interleukin-2 in each MOF by measuring the concentration of protein in the supernatants. For BSA, Catalase and Myoglobin MOFs, supernatants were separated from the solids and concentrated using a rotavap. An aliquot of 20 µL was taken from the concentrated supernatant and mixed into a solution of 980 µL Bradford reagent 1x (standard assay). Absorbance measurements were obtained from each sample at 595nm using a NanoDrop™ 2000. Concentrations were calculated using calibration curves for each protein. For myoglobin, the encapsulation efficiency was determined directly by measuring the absorbance of the supernatant at 408 nm. For interleukin-2, the supernatant was concentrated with a centrifugal filter (Amicon Ultra 0.5 ml, MWCO 30K) and diluted to 150 µL. The 150 µL of supernatant was mixed with 150 µL of Bradford reagent (Microassay), and the absorbance at 595nm was analyzed with a NanoDrop™ 2000 using myoglobin as the standard.

	BSA	Catalase	Myoglobin	Interleukin-2
γ-CD-MOFs:	95%∓3%	90%∓2%	95%∓3%	90%∓2%
β-CD-MOFs:	85%∓5%	94%∓4%	95%∓4%	95%∓3%

Table S1. Encapsulation Efficiency of BSA, Catalase, Myoglobin, and Interleukin-2 in γ-CD-MOFs and β-CD-MOFs.

Release profile*.*

BSA was encapsulated in three different CD-MOF systems: β-CD-MOFs, γ-CDMOF, and a hybrid γ-/ β-CD-MOF. 50 mg of each protein @ CDMOF were incubated in 4 ml of Milli-Q water, and the release percentage was calculated by taking an aliquot of 20 µl every 30 minutes and using it with 980 µL of Bradford reagent 1x. The absorbance was calculated at 595 nm.

Structural Characterization

Samples analyzed by SEM were sputter-coated with ~5 nm of iridium (Quorum Q150T) and imaged by an FEI Magellan 400 XHR system. Secondary electron images were acquired with an

accelerating voltage of 10 kV, using a lens detector operating in immersion mode. Using an Ultima X-ray diffractometer, PXRD patterns were acquired using X-rays generated at 40 kV and 44 mA with Cu Kα irradiation.

No.	2-theta γ-CD-MOF	d-spacing (ang.)	2-theta β-CD-MOFs	d-spacing (ang.)
1	4.040(7)	21.85(4)	4.469(13)	19.76(6)
2	5.680(5)	15.547(13)	6.123(4)	14.423(11)
3	6.970(5)	12.672(9)	8.865(12)	9.966(14)
4	8.020(9)	11.015(13)	9.718(16)	9.093(15)
5	8.960(10)	9.862(11)	10.622(4)	8.322(3)
6	9.86(3)	8.96(2)	11.462(8)	7.714(6)
7	11.430(14)	7.735(9)	12.396(13)	7.135(7)
8	12.030(12)	7.351(8)	12.561(4)	7.041(2)
9	13.370(5)	6.617(2)	14.622(10)	6.053(4)
10	16.240(18)	5.454(6)	15.308(7)	5.783(3)
11	16.640(11)	5.323(4)	16.040(18)	5.521(6)
12	17.040(19)	5.199(6)	16.77(3)	5.282(8)
13	17.51(4)	5.061(10)	17.009(8)	5.208(3)
14	18.360(19)	4.828(5)	17.416(12)	5.088(4)
15	19.62(2)	4.521(5)	17.710(17)	5.004(5)
16	20.600(12)	4.308(3)	18.022(13)	4.918(3)
17	21.030(17)	4.221(3)	18.562(12)	4.776(3)
18	22.530(12)	3.943(2)	18.92(2)	4.686(6)
19	23.180(18)	3.834(3)	19.552(11)	4.536(3)
20	23.950(17)	3.713(3)	20.544(17)	4.320(4)
21	26.13(4)	3.408(6)	20.99(6)	4.228(12)

Table S2. d-spacing of γ-CD-MOF and β-CD-MOF, obtained from the PXRD analysis.

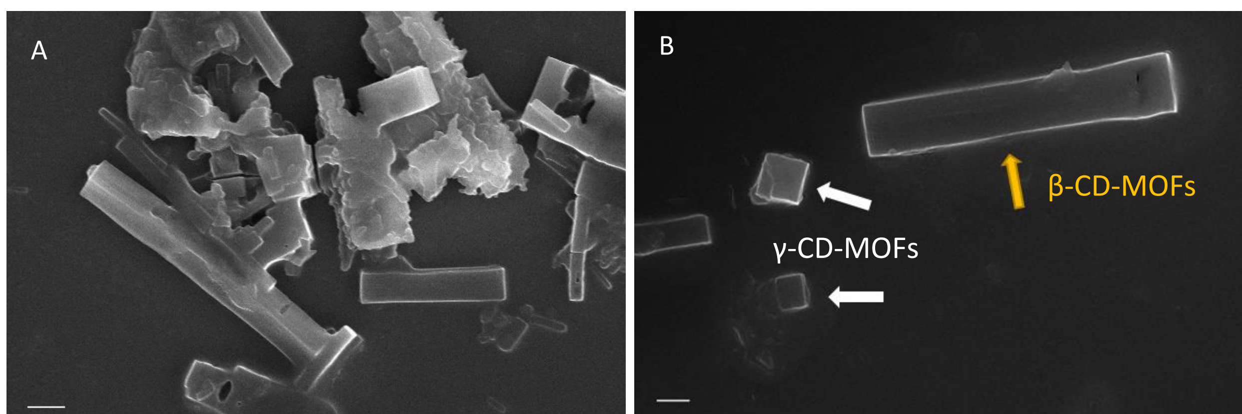

Figure S1. SEM pictures of hybrid γ-/ β- CD-MOFs. (a) Mixture of Irregular aggregates and CDMOFs (b)White arrows indicated crystal with identical shape observed for γ-CD-MOFs crystals. Yellow arrows indicate crystals with an identical shape observed for β-CD-MOFs crystals. Scale Bar 2μm

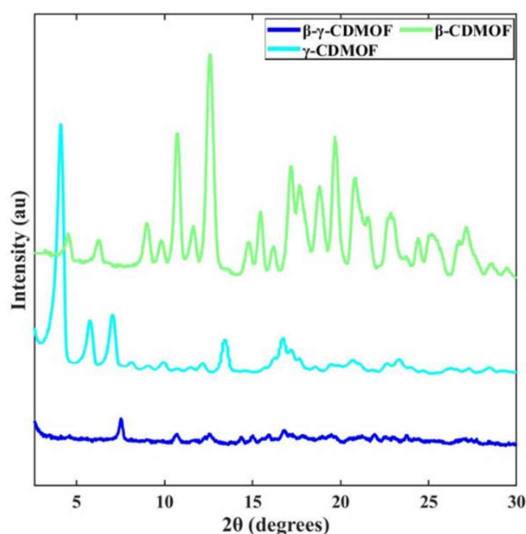

Figure S2. PXRD of β-CD-MOF (green), γ-CD-MOF (light blue), and the hybrid β-/ γ-CD-MOF.

Catalase activity assay

For the preparation of 100 ml FOX reagent: the solution consisted of 90% methanol and 10% Milli-Q water. 139 µL of sulfuric acid 98%, 9.8 mg of ferrous ammonium sulfate and 7.6 mg of xylenol orange were added in this order to the solution. The solution was left stirring for one hour until the complete dissolution of all the compounds. Catalase and Catalase@CD-MOFs were added into two vials containing 2 ml of PBS 1x to obtain a final concentration of 40 nM for the enzyme. 5 µL of 30% hydrogen peroxide was added for every 1 ml of enzyme solution. The stirring was set at 300 rpm. The assay consisted in taking an aliquot of 50 µL of the Catalase and the

hydrogen peroxide solutions every 30 seconds and subsequently adding to 950 μL of FOX reagent. The absorbance was measured at 560 nm.

In vitro T cell proliferation assay

A single cell suspension was prepared from the spleen and lymph nodes of a 9-weeks-old C57BL/6J male mouse (Jackson laboratories stock #000664) by mechanical disruption followed by red blood cell lysis with ACK buffer. Cells were then labelled with 5μM CellTrace Violet (BioLegend C34557) and plated $2x10^5$ per well in T cell culture medium (RPMI, 10% FBS, HEPES 15mM, non-essential aminoacids, sodium pyruvate 1mM and β-mercaptoethanol 55μM) in a 96-well flat bottom plate. Lymphocytes were stimulated with 50ng/ml CD3ε antibody (clone 145-2C11 BioLegend) and varying concentrations of either free Interleukin-2, Interleukin-2@CD-MOFs, or myoglobin@CD-MOFs then incubated at 37° Celsius.

After 72 hours, T cell proliferation was assessed through flow cytometry by staining cells with Zombie Red 1:400 (BioLegend), FcBlock 5 μg/ml (clone S17011E BioLegend), anti-CD4 APC 1 μg/ml (clone RM4-5 BioLegend), and anti-CD8 BV785 1 μg/ml (clone 53-6.7 BioLegend). Cytometry data was then processed with FCS Express and quantified with GraphPad Prism. The fold expansion of $CD4^+$ and $CD8^+$ T cells in each sample was calculated by dividing the count of T cells from all generations by the number of progenitor cells. Considering that the number of cells in each division peak (n) originated from $n/2^{division}$ progenitor cells,[1] we calculated the number of progenitors as the sum of progenitor cells that originated each peak of fluorescence. Statistical analysis was performed by Student's t test, and p values lower than 0.05 were considered significant.

1 A. L. Givan, J. L. Fisher, M. G. Waugh, N. Bercovici and P. K. Wallace, in *Flow Cytometry Protocols*, eds. T. S. Hawley and R. G. Hawley, Humana Press, Totowa, NJ, 2004, pp. 109–124.