Superoxide dismutase transcellular shuttle constructed from dendritic MOF and charge reversible protein derivatives

Wei Wang[a,b,¶], *Sudong Wu*[c,d,¶], *Jingyun Wang*[a,b], *Zhen Li*[e], *Hongyan Cui*[b], *Shuseng Lin*[b], *Jingyi Zhu*[b], *Qixian Chen*[a,b,*]

[a]State Key Laboratory of Fine Chemicals, Dalian University of Technology, No. 2 Linggong Road, Dalian 116024, China

[b]School of Life Science and Biotechnology, Dalian University of Technology, No. 2 Linggong Road, Dalian 116024, China

[c]Department of Materials Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China

[d]Academy for Advanced Interdisciplinary Studies, Southern University of Science and Technology, Shenzhen 518055, China

[e]College of Pharmacy, Dalian Medical University, No. 9 West Section Lvshun South Road, Dalian 116044, China

¶These two authors contributed equally to this work (W. W and S. W.).

Corresponding Authors

*E-mail: qixian@dlut.edu.cn (Q. Chen)

S1. Amino acids sequence of superoxide dismutase [Cu-Zn]

ATKAVCVLKG DGPVQGIINF EQKESNGPVK VWGSIKGLTE GLHGFHVHEF GDNTAGCTSA GPHFNPLSRK HGGPKDEERH VGDLGNVTAD KDGVADVSIE DSVISLSGDH CIIGRTLVVH EKADDLGKGG NEESTKTGNA GSRLACGVIG IAQ

S2. Chemical structures of PGMA(EA) and MOF-PGMA(EA)

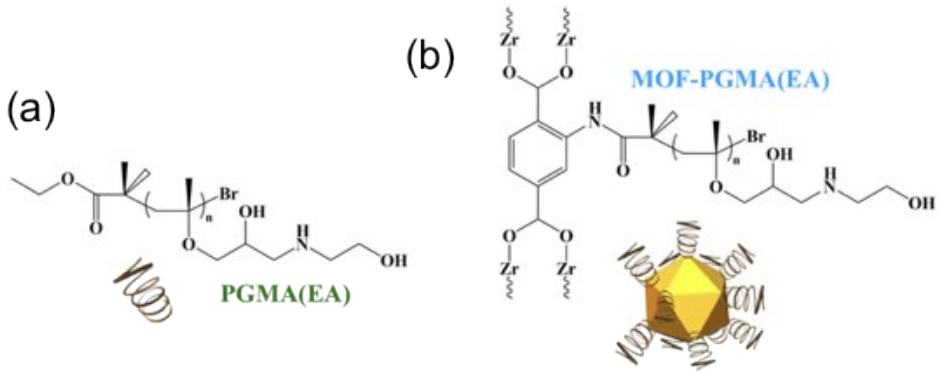


Fig. S1 Chemical structures and schematic illustration of the dendritic catiomer of MOF-PGMA(EA) and the linear catiomer of PGMA(EA). (a) chemical structure of PGMA(EA); (b): chemical structure of MOF-PGMA(EA).

S3. Synthetic procedures and characterization of MOF-PGMA(EA)

a): Synthesis of NH2-UiO-66 (MOF)

The synthesis of NH_2-UiO-66 was performed according to a solvothermal approach. In brief, 2-amino-benzenedicarboxylic acid (0.248 g, 1.372 mmol) and $ZrCl_4$ (0.320 g, 1.372 mmol) were dissolved in 80 mL DMF in Teflon-lined stainless-steel autoclave. Furthermore, 1.236 mL water (68.7 mmol, 50 equiv. to $ZrCl_4$) was added to the above DMF solution under stirring. The mixture was sealed for reaction in an oven at 120 °C for 24 h. After cooling down to room temperature, the precipitate was obtained by centrifugation and washed with a mixture of DMF and methanol. The obtained crystal was immersed in methanol for 24 h, rinsed with methanol, and dried under reduced pressure for 24 h at 80 °C.

b): Synthesis of the Br-functionalized NH2-UiO-66 (UiO-BiBB) (Br-MOF)

UiO-BiBB was obtained by conjugation of the yielded NH_2-UiO-66 with BiBB. In brief, 0.30 g NH_2-UIO-66 (containing 1 mmol $–NH_2$) was dispersed in 20 mL anhydrous THF by sonication. TEA (209 mL, 1.5 mmol) and

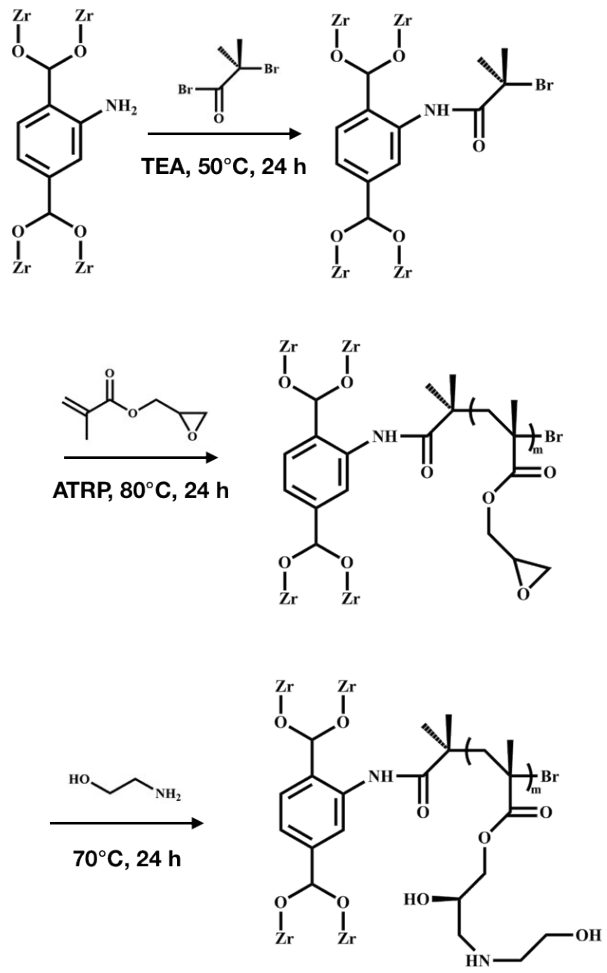
BiBB (62 mL, 0.5 mmol) were dissolved in 5 mL THF. The TEA solution was injected into the NH_2-UiO-66 suspension under stirring, followed by titration of BiBB solution at room temperature under stirring. The reaction was conducted at 50 °C for 24 h. The product was washed with THF and methanol. The resulting UiO-BiBB was collected and incubated in methanol for 24 h, washed with methanol, and dried under reduced pressure for 24 h at 40 °C.

c): Synthesis of UiO-PGMA (MOF-PGMA)

The polymerization of PGMA from the yielded UiO-BiBB was based on atom transfer radical polymerization approach. In brief, 73.4 mg of UiO-BiBB (ca. 0.09 mmol of a-bromoisobutyryl group) (1 equiv.) was dispersed in 35 mL of anhydrous THF by sonication. Bipyridyl (21.08 mg, 0.135 mmol) (1.5 equiv.), GMA (0.833 mL, 6.3 mmol) (70 equiv.), and CuBr (12.9 mg, 0.09 mmol) (1 equiv.) were added to the above THF solution. Note that the reaction mixture was subjected to degassing treatment and kept under a nitrogen atmosphere throughout the reaction. Atom transfer radical polymerization of PGMA segment was conducted at 80 °C for 24 h. After cooling down to room temperature, the yielded solution was centrifuged, and the supernatant was condensed and precipitated in diethyl ether. The final product was dried under reduced pressure for 24 h at 40 °C.

d): Synthesis of UiO-PGMA(EA) [MOF-PMGA(EA)]

The yielded UiO-PGMA was schemed to proceed aminolysis to create hyper-charged UiO-PGMA(EA). In brief, 15 mg UiO-PGMA was dissolved in DMSO (4 mL). Furthermore, 1.5 mL of ethanolamine was added to the above DMSO solution. The reaction was conducted under a nitrogen atmosphere at 70 °C under stirring for 24 h. The crude product was purified by dialysis (Spectra/Por RC, MWCO: 7 kDa) against deionized water for 48 h, followed by lyophilization to obtain UiO-PGMA(EA) as white solid. The resulting product was transferred to ^{1}H-NMR measurement (Figure S2). Note that the protons of benzyl rings in the MOF core of UiO-PGMA(EA) was not visible in ^{1}H-NMR spectrum due to insolubility of MOF core in D_2O.



Scheme S1 Synthetic scheme of preparation of MOF-PGMA(EA).

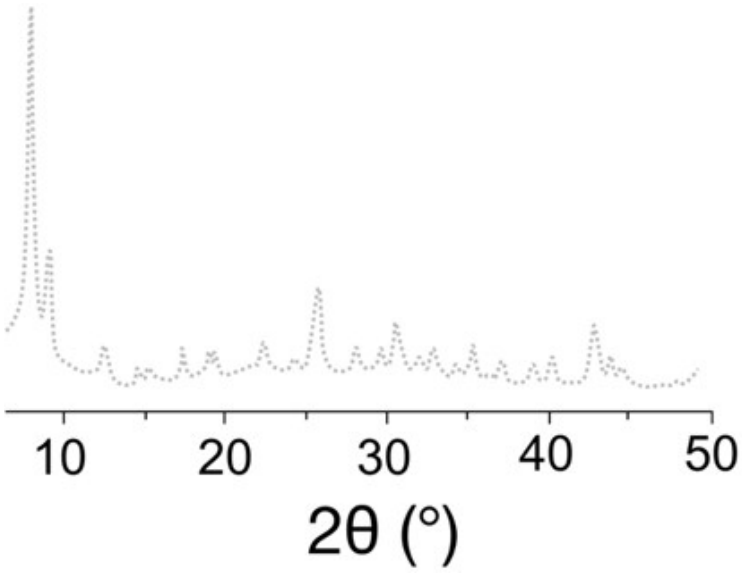


Fig. S2 XRD spectrum of MOF-PGMA(EA).

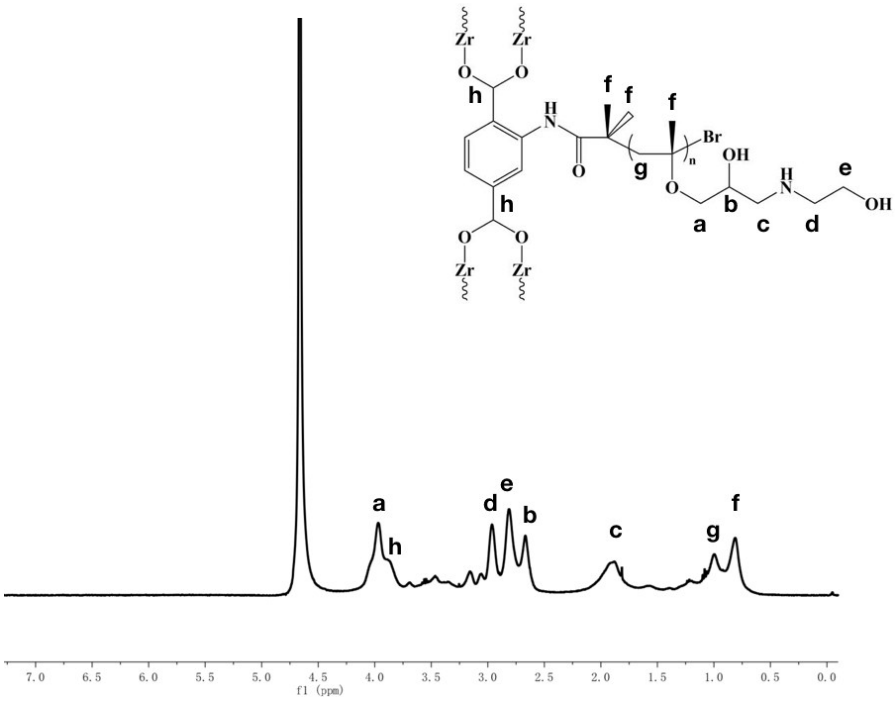


Fig. S3 ^{1}H-NMR spectrum of MOF-PGMA(EA) in D_2O.

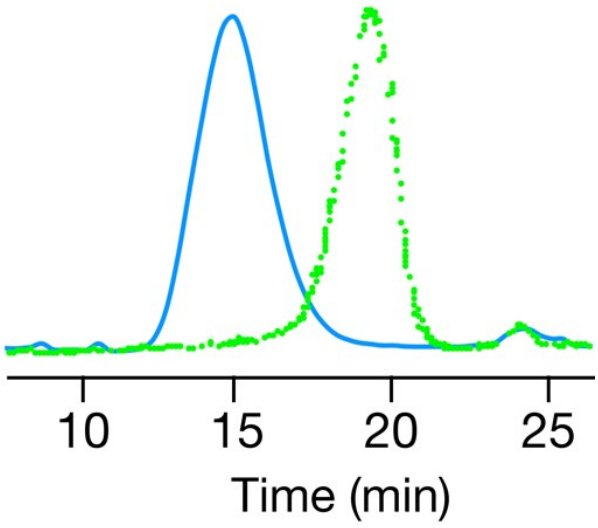


Fig. S4 GPC traces of the linear control catiomer of PGMA(EA) (green) and MOF-PGMA(EA) (blue).

Table S1 Chemical descriptions of MOF-PGMA(EA) and PGMA(EA).

Catiomers	Numbers of PGMA(EA) segments per catiomer	Approximate polymerization degree of PGMA(EA) segment	Total numbers of amino groups per catiomer
PGMA(EA)	1	41	41

MOF-PGMA(EA)	12[a]	35.6[a]	427[b]

[a]Theoretical assumption by considering the number of available ligands for subsequent ATRP.

[b]Experimental calculation based on quantification of total amine groups by fluorescamine assay and quantification of zirconium composition by ICP-MS measurement.

S4. Cellular uptake activity by fluorescence microscopy

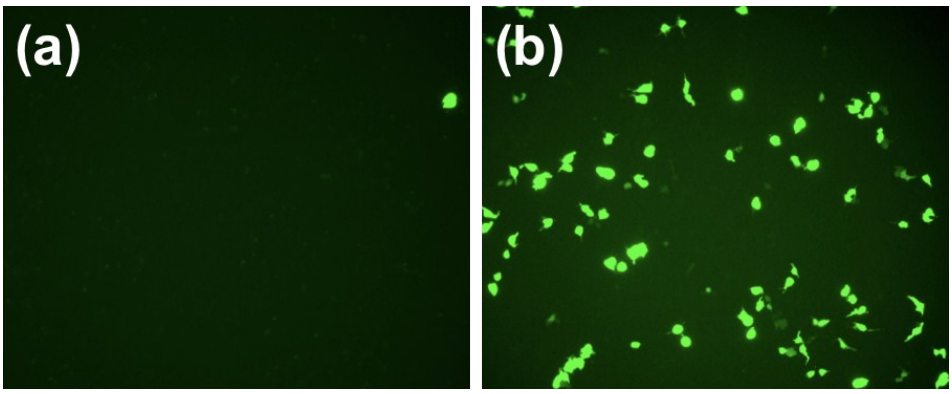


Fig. S5 Cellular internalization of the native SOD and MOF-PGMA(EA)/SOD-60, wherein SOD (derivatives were stained into green). (a): the native SOD; (b) MOF-PGMA(EA)/SOD-60.

S5. Comparison study with commercial protein transfection reagents

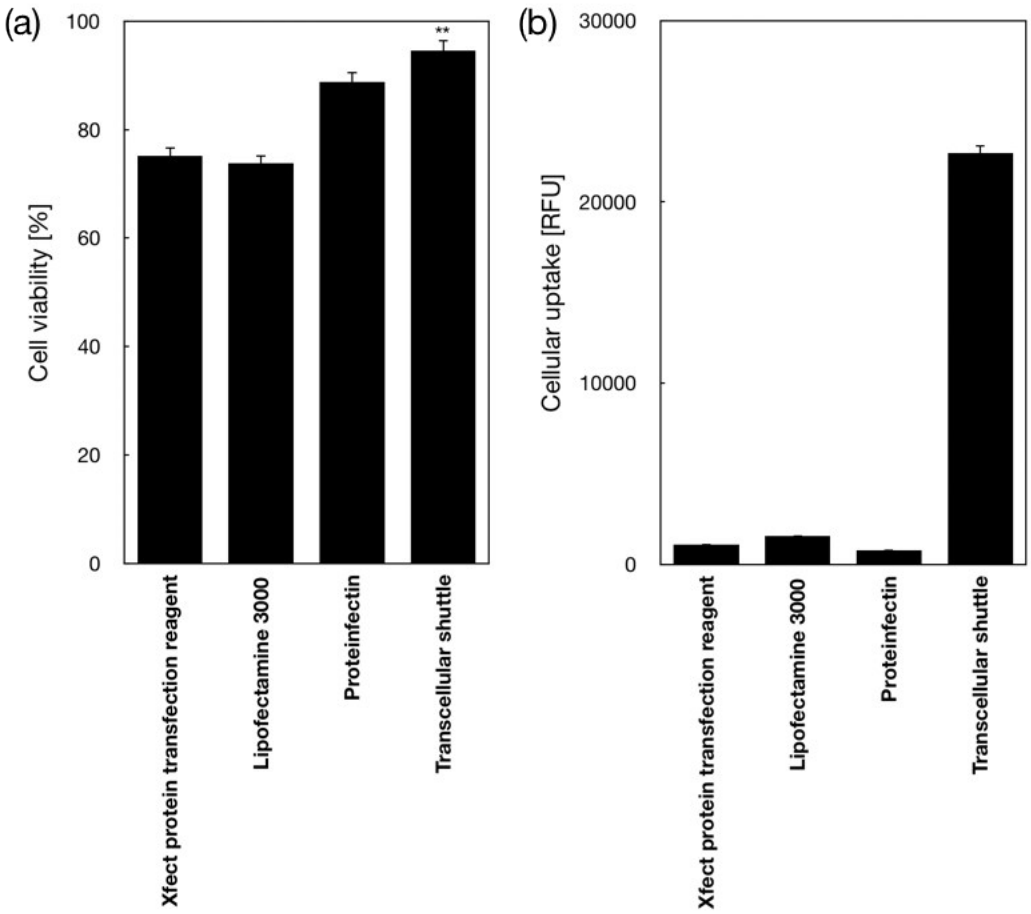


Fig. S6 Cell viabilities (a) and cellular uptake activities (b) of our proposed transcellular shuttle in comparison with the commercial protein transfection agent.

S6. *In vitro* FRET efficiency of MOF-PGMA(EA)/SOD-60 upon incubation at acidic pH 5

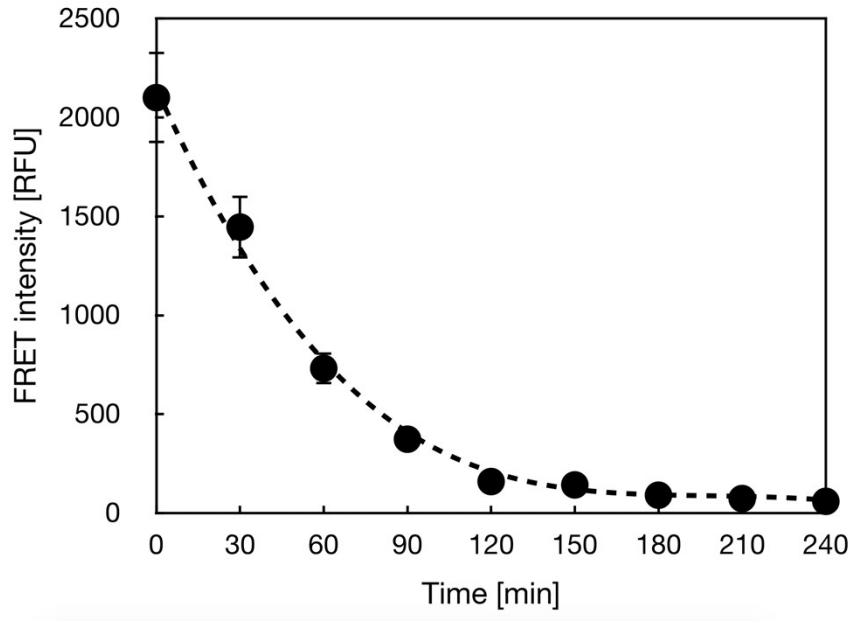


Fig. S7 FRET intensities of MOF-PGMA(EA)/SOD-60 upon acidic pH (5) incubation as a function of incubation time, wherein MOF-PGMA(EA) and SOD-60 were labeled by Cy3 and Cy5, respectively.