SUPPLEMENT: Conjugates of Superoxide Dismutase 1 with Amphiphilic Poly(2-oxazoline) Block Copolymers for Enhanced Brain Delivery: Synthesis, Characterization and Evaluation in vitro and in vivo

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Table S1. Acid precipitation of ^{125}I-SOD1-(cc)-P(EtOx-b-BuOx) by brain and serum

Time (min)	Serum (%)	Brain (%)	Brain (%)[a]
15	104.62 ± 0.35	96.94 ± 1.27	ND
60	99.13 ± 2.03	84.13 ± 2.75	57.57 ± 4.31
240	77.04 ± 12.08	43.76 ± 3.43[b]	ND

[a] $P<0.005$ compared between 15 min and 240 min group (n=2), no statistical significant difference between 15 min and 60 min group.

[b] Vascular space of brain was washed out before collection.

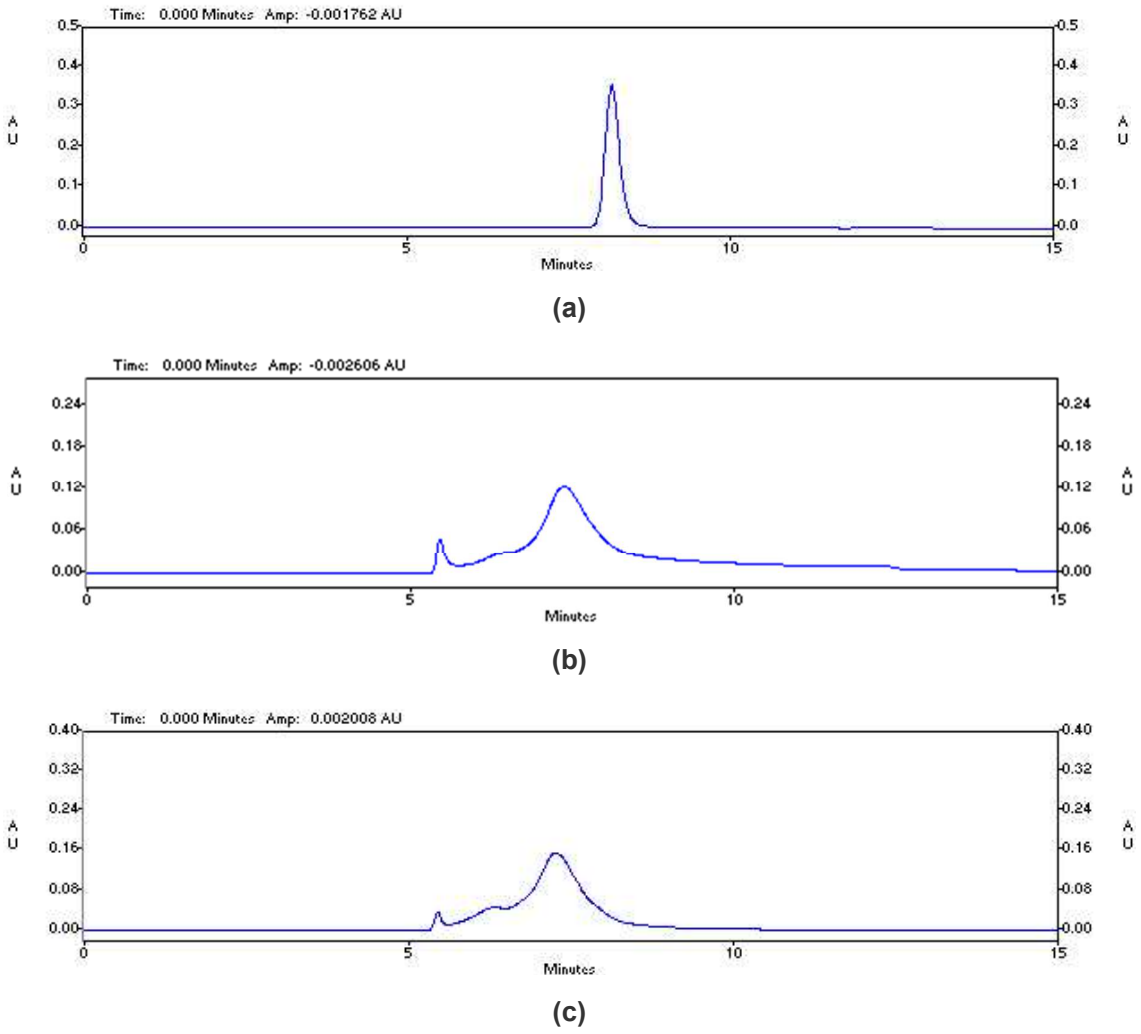


Figure S1. Representative size-exclusion chromatographic profiles of (a) SOD1, (b) SOD1-(cc)-P(MeOx-*b*-BuOx) and (c) SOD1-(cc)-P(EtOx-*b*-BuOx). UV absorbance was detected at 220 nm. The mobile phase was methanol (5%) and pH 6.8, 0.1 M NaH_2PO_4, 0.2 M NaCl buffer (95%).

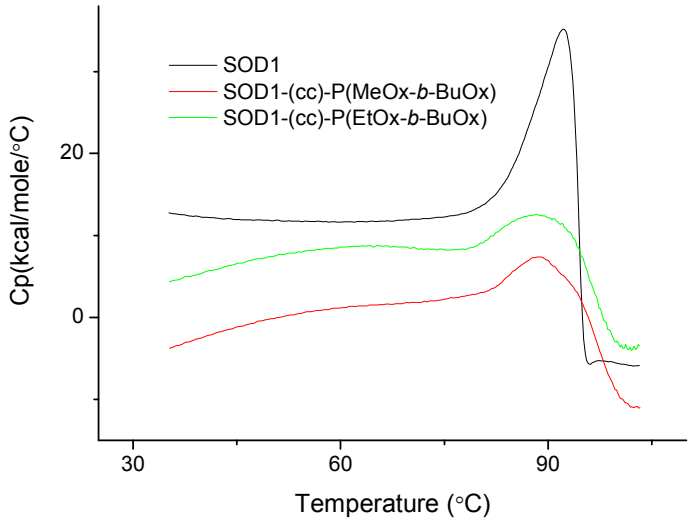


Figure S2. Melting curves of SOD1 or SOD1-POx solution was measured by Microcal DSC. SOD1 or SOD1-POx was dissolved in PBS (pH 7.4) at the concentration of 2.0 mg/mL and used for the measurement. Melting curves were obtained from 10°C to 110°C in 0.2°C increments.

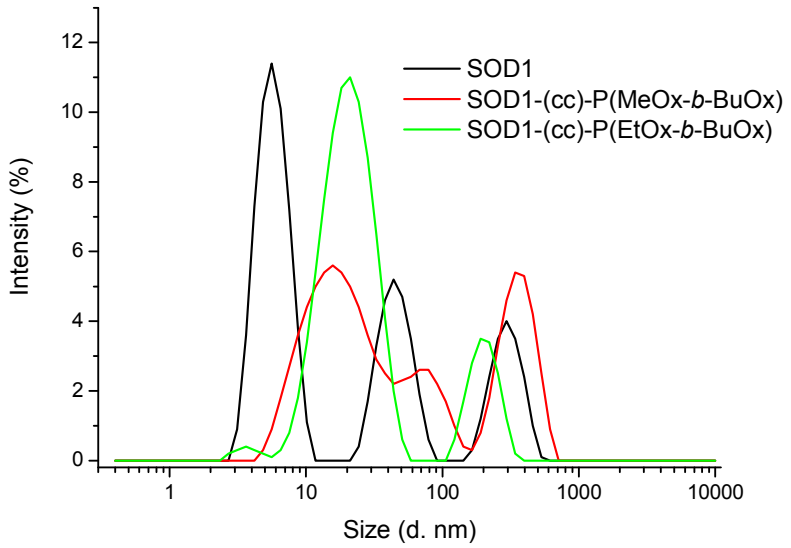


Figure S3. Intensity-based size distributions of the particles in SOD1 and SOD1-POx solutions determined by DLS at 25 °C. All samples were dissolved in PBS (pH 7.4) at the concentration of 0.5 mg/mL (determined by MicroBCA assay) and sterile filtered (450 nm filter) prior to the measurements. These intensity-based size distributions are more heavily "weighted" towards the particles of larger size and therefore are more useful for illustrating the presence of larger aggregates in the samples rather for determining the relative amounts of these aggregates.

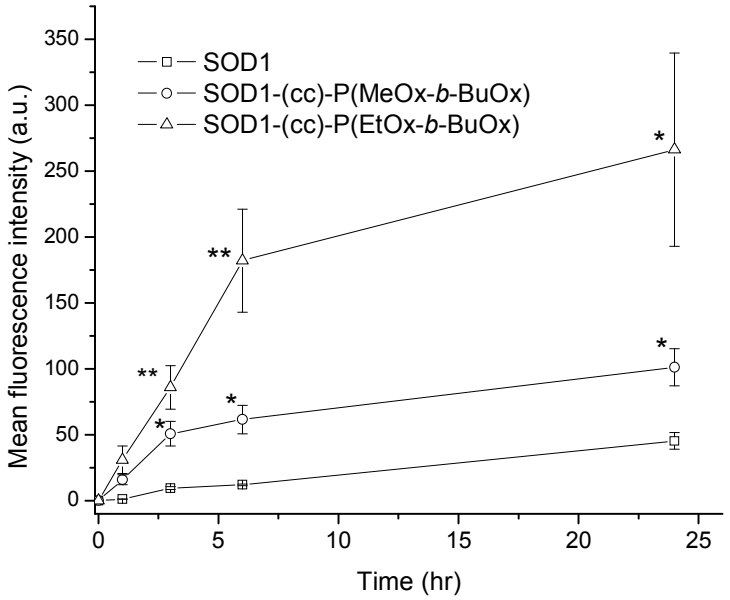
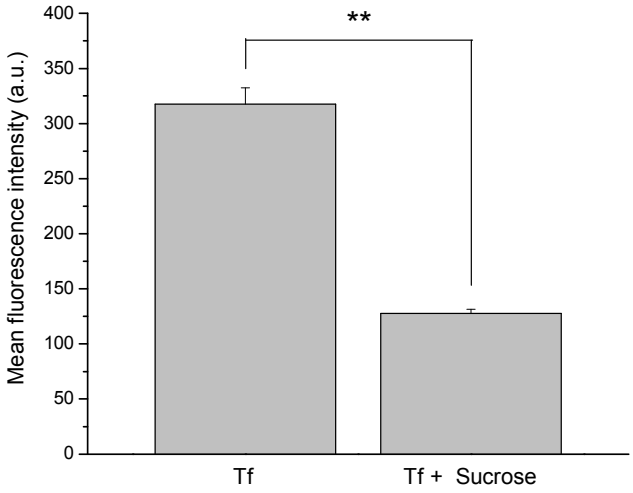
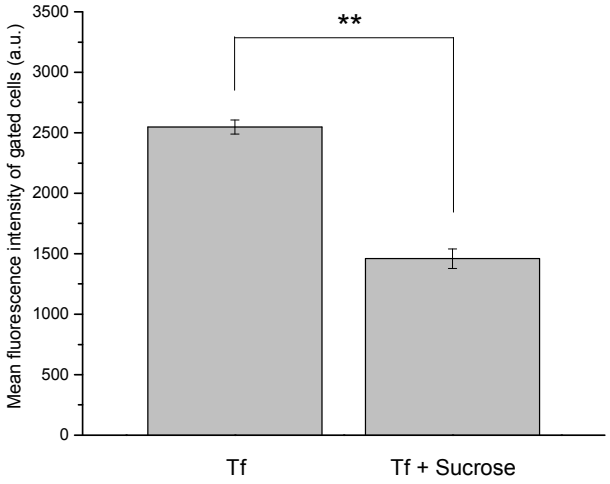


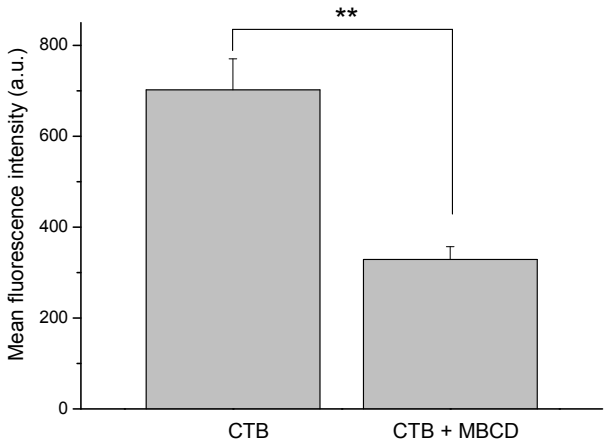
Figure S4. Quantitative analysis of the cellular uptake of fluorescently-labeled SOD1 and SOD1-POx obtained by CLSM. Cells were incubated with 80 μg/mL of SOD1 or SOD1-POx for 1 h-24 h in serum-free conditions and imaged by CLSM. Data are presented as means ± SEM (n=10-15), * $p<0.05$ and ** $p<0.01$.


**
Mean fluorescence intensity (a.u.)
400
350
300
250
200
150
100
50
0
Tf
Tf + Sucrose

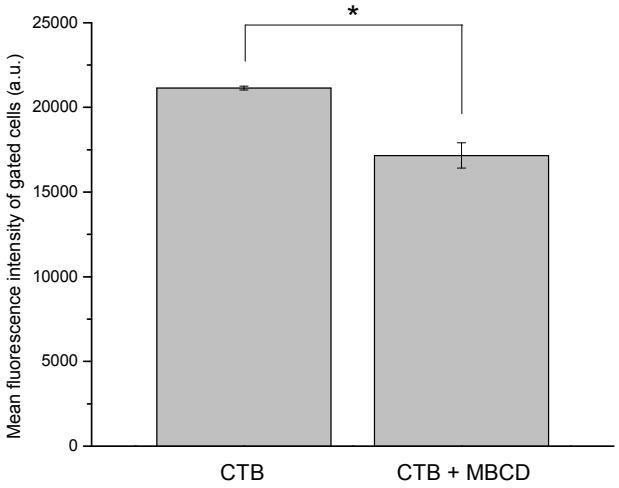
(a)


**
Mean fluorescence intensity of gated cells (a.u.)
3500
3000
2500
2000
1500
1000
500
0
Tf
Tf + Sucrose

(b)



(c)



(d)

Figure S5. Inhibition of the cellular uptake of (a and b) Tf by hypertonic sucrose and (c and d) CTB by MBCD as determined by (a and c) CLSM and (b and d) flow cytometry. Cells were pre-treated with 0.4 M of hypertonic sucrose or 4 mM of MBCD for 30 min and then co-incubated with 10 μg/mL of Tf or 10 μg/mL of CTB for 6 h. The quantitative mean fluorescence intensity was analyzed and compared with non-treated controls. Data are presented as means ± SEM (n=10-15 for CLSM and n=3 for flow cytometry), * $p<0.05$ and ** $p<0.01$.

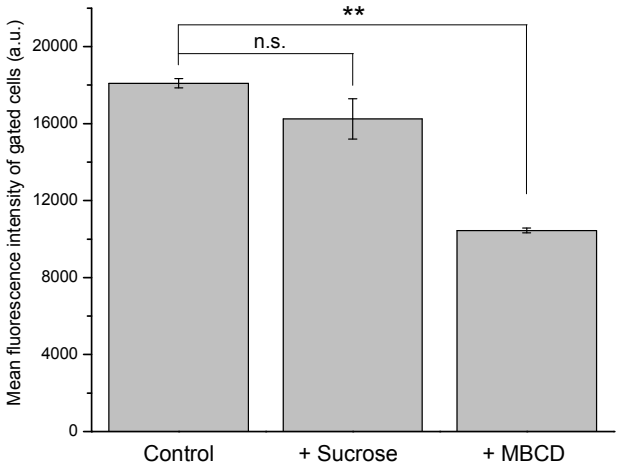


Figure S6. Inhibition of the cellular uptake of SOD1-(cc)-P(EtOx-*b*-BuOx) by hypertonic sucrose and MBCD as determined by flow cytometry. Cells were pre-treated with hypertonic 0.4 M sucrose or 4 mM MBCD for 30 min and then co-incubated with 80 µg/mL of SOD1-(cc)-P(EtOx-*b*-BuOx) for 6 h. The quantitative mean fluorescence intensity of gated cells was analyzed and compared with non-treated controls. Data are presented as means ± SEM (n=3), ** $p<0.01$.

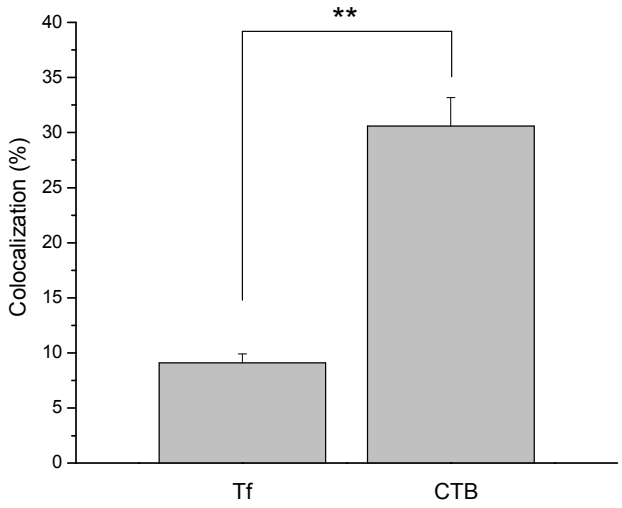
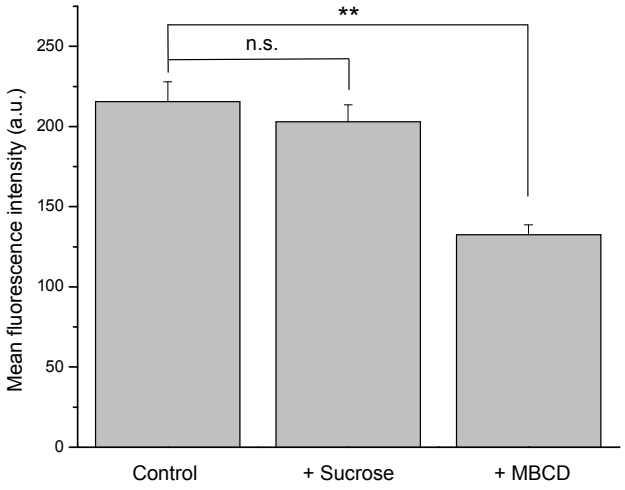
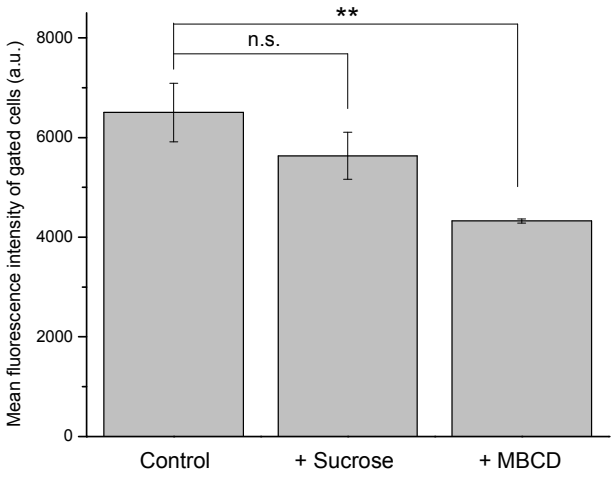


Figure S7. Colocalization of fluorescently-labeled P(EtOx-*b*-BuOx) with specific endocytosis markers Tf and CTB. Cells were co-incubated with 50 µg/mL of fluorescently-labeled P(EtOx-*b*-BuOx) and Alex Fluor® 488-conjugated Tf or CTB for 2 h. Colocalization of P(EtOx-*b*-BuOx) with Tf or CTB was analyzed by Image J. Data are presented as means ± SEM (n=10-15), ** $p<0.01$.



(a)



(b)

Figure S8. Inhibition of the cellular uptake of free P(EtOx-*b*-BuOx) by hypertonic sucrose and MBCD as determined by (a) CLSM and (b) flow cytometry. Cells were pre-treated with 0.4 M of hypertonic sucrose or 4 mM of MBCD for 30 min and then co-incubated with 50 µg/mL of fluorescently-labeled P(EtOx-*b*-BuOx) for 1 h. The quantitative fluorescence intensity was analyzed and compared with non-treated controls. Data are presented as means ± SEM (n=10-15 for CLSM and n=3 for flow cytometry), ** $p<0.01$.

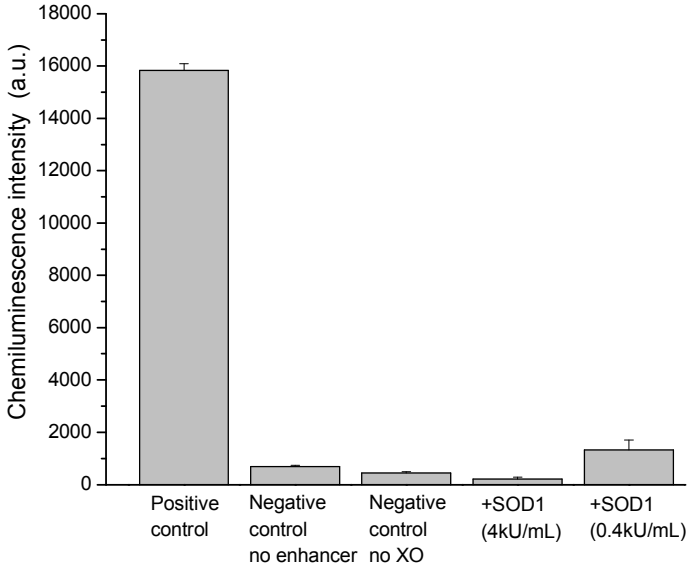


Figure S9. Validation of luminol-based chemiluminescence assay. HX-XO system was used as the superoxide source. Data are presented as means ± SEM (n=3).

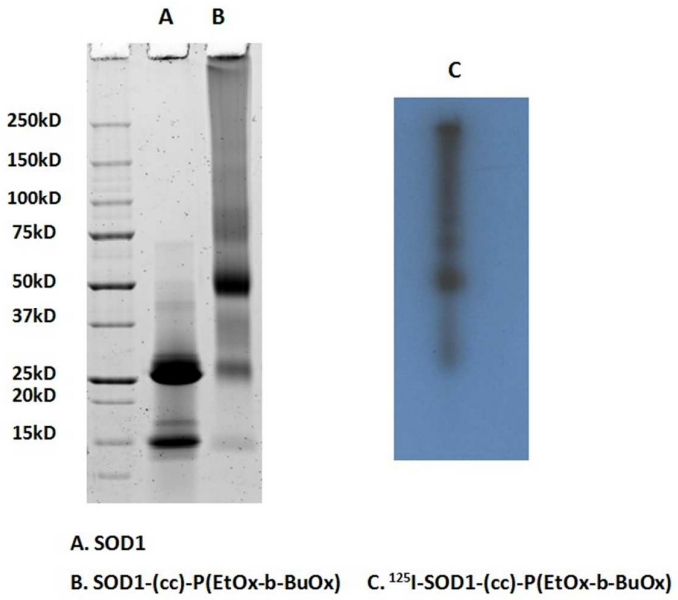


Figure S10. SDS-PAGE of SOD1 and SOD1-(cc)-P(EtOx-*b*-BuOx) before (left) and after (right) iodination.