SUPPLEMENTARY METHODS

Enzyme Activity. Activities were measured by monitoring the change in absorbance at 240 nm (catalase) and 420 nm (SOD1) using Lambda 25 UV VIS Spectrophotometer (Perkin Elmer Instruments, Waltham, MA or Ultrospec™ 2100 *pro*, Amersham Biosciences). As an example of SOD1 assay: 1 mL 60 mM Tris (pH 8.2) + 1 mM DTPA, 2 µL pyrogallol (0.4 M) was added into both cuvettes, 1-6 µL SOD1 (1 mg/mL) was then added into reference cuvette. As an example of catalase assay: 1 mL 60 mM phosphate buffer (pH 7.4), 1-15 µL H_2O_2 (5 mM – 8 M) and 1-6 µL catalase (0.1 – 5 µM) was added into sample cuvette. Catalase activity was evaluated by monitoring the decrease at A_{240} resulting from the decomposition of H_2O_2 (extinction coefficient at 240 nm: 43.6 $M^{-1}cm^{-1}$). As a quantitative SOD1 activity assay, enzyme concentration at which 50% inhibition of $O_2^{-\cdot}$ dismutation occurred was measured using luminol/xanthine/xanthine oxidase (Femtomaster FB 12, Zylux corporation, USA) and riboflavin/Nitro Blue Tetrazolium (NBT) assays.[1,2] The initial activity was 3780 U/mg protein for SOD1 and 2000 or 46500 U/mg protein for catalase (as indicated by the manufacturer).

SUPPLEMENTARY TABLES

Table S1. Number-average diameter of selected nanozymes measured using AFM

Sample	Linker	Small particles, nm	Big particles, nm
SOD1	-	11 ± 1	23 ± 3
SOD1/PEI-PEG; cl*	BS^3	21 ± 1	45 ± 2
SOD1/PEI-PEG; cl	EDC/S-NHS	21 ± 2	45 ± 3
SOD1/pLL_{10}-PEG; cl	EDC/S-NHS	21 ± 1	40 ± 3
SOD1/pLL_{50}-PEG; cl	EDC/S-NHS	24 ± 3	45 ± 2
catalase (cat)	-	20 ± 1	100 ± 3
cat/PEI-PEG; cl	GA	25 ± 2	41 ± 3
cat/PEI-PEG; cl	BS^3	24 ± 2	50 ± 3
SOD1-cat/PEI-PEG; cl	EDC/S-NHS	24 ± 1	44 ± 3
SOD1-cat/pLL_{10}-PEG; cl	EDC/S-NHS	22 ± 1	34 ± 2

*cross-linked

SUPPLEMENTARY FIGURES LEGEND

Figure S1. Dependence of the reaction rates on enzyme concentration. (A) $O_2^{-\cdot}$ dismutation catalyzed by SOD1 monitored as inhibition of pyrogallol autoxidation (decrease in A_{420}) measured in 60 mM Tris (pH 8.2) containing 0.8 mM pyrogallol at 25 °C. (B) Decomposition of hydrogen peroxide in the presence of native catalase (□) and GA cross-linked catalase/PEI-PEG (*Z*=1) (■) measured in 60 mM phosphate buffer (pH 7.4) containing 8 mM H_2O_2 at 25 °C.

Figure S2. Dependence of the reaction rate of hydrogen peroxide decomposition on substrate concentration (double reciprocal coordinates) in the presence of native catalase (□) and GA cross-linked catalase/PEI-PEG (*Z*=1) (■) at a catalase concentration of 4 nM measured in 60 mM phosphate buffer (pH 7.4) at 25 °C.

Figure S3. Quantitative estimation SOD1 activity using (A) riboflavin/NBT in 50 mM KH_2PO_4 (pH 7.8) buffer containing 2 μM riboflavin and 57 μM NBT and absorbance was measured at 560 nm. (B) Luminol/xanthine/xanthine oxidase assay in 50 mM bicarbonate buffer (pH 10.2) containing 0.33 mM luminol, 0.1 mM xanthine and 1 mM xanthine oxidase at 25 °C. Native SOD1 (□) and GA cross-linked SOD1/PEI-PEG (*Z*=1) (■).

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2. Janknegt PJ, Rijstenbil JW, van de Poll WH, Gechev TS, Buma AG. A comparison of quantitative and qualitative superoxide dismutase assays for application to low temperature microalgae. J Photochem Photobiol B 2007;87:218-26.