Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information

Evaluation of exogenous therapeutic protein activity under confinement and crowding effects

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SUPPLEMENTARY FIGURES

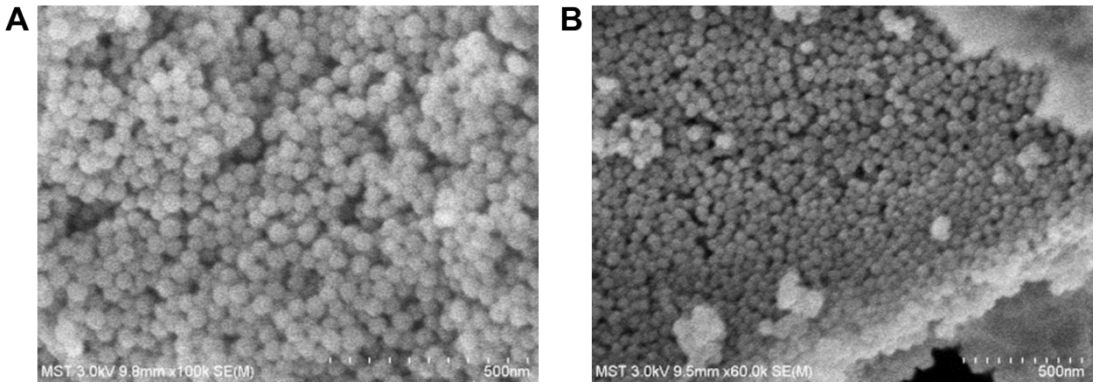

Fig. S1 SEM images of (A) BS-NPs and (B) HRP@BS-NPs in a broader version.

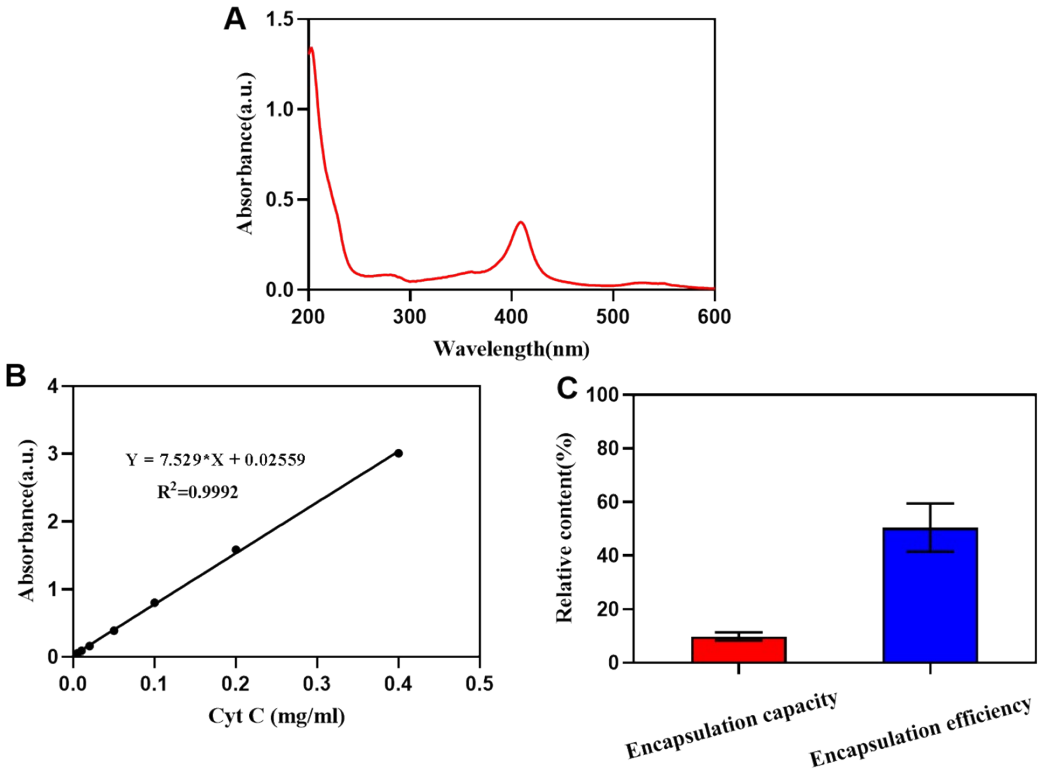

Fig. S2 Quantification of Cyt C protein in the confined Cyt C@BS-NPs. (A) UV-vis spectra of the Cyt C standard solution. (B) Linear plot of UV-vis absorbance value at 409 nm *versus* the different concentrations of Cyt C standard solutions. (C) The corresponding encapsulation capacity and encapsulation efficiency of Cyt C into Cyt C@BS-NPs.

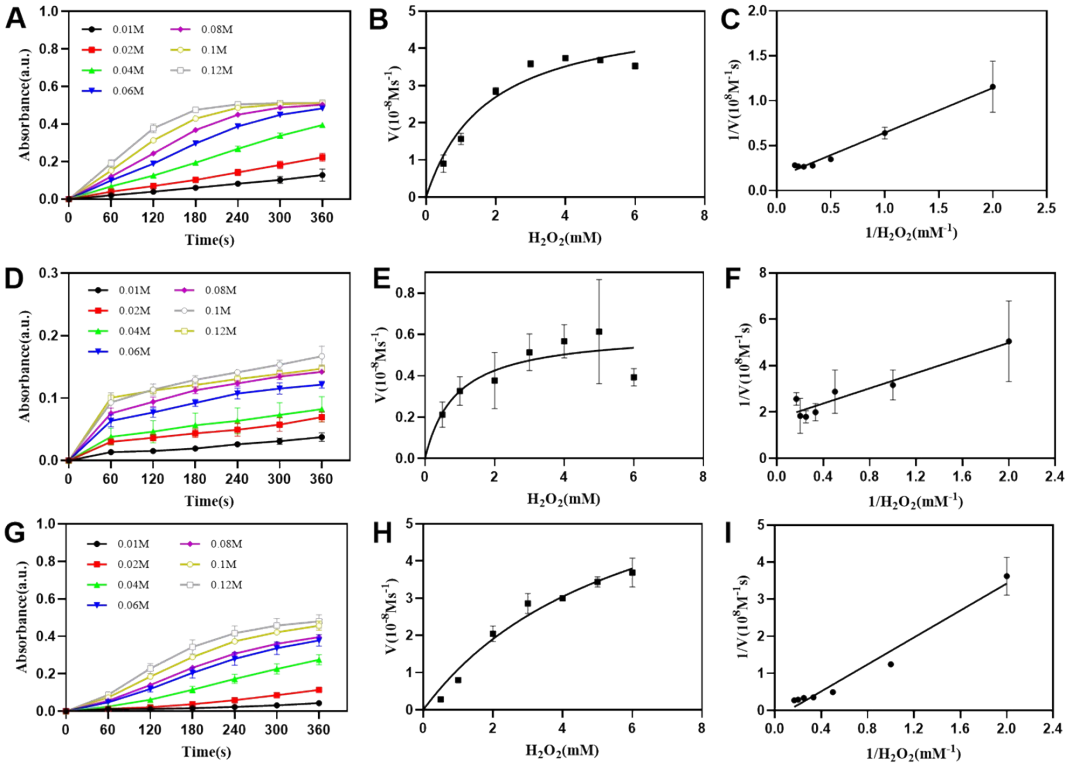

Fig. S3 Cyt C protein activity characterization under different conditions. (A, D, G) Time-dependent absorbance changes caused by the oxidation product of oxTMB produced from TMB colorimetric assay under different environments: A) free solution, D) confined Cyt C@BS-NPs, and G) PEG_{4000}-induced crowding. (B, E, H) Steady-state kinetic assay under different environments: B) free solution, E) confined Cyt C@BS-NPs, H) PEG_{4000}-induced crowding, and (C, F, I) are their corresponding double-reciprocal plots, respectively.

Table S1. Detailed kinetic data of Cyt C under different environments.

Catalyst	Substrate	K_m / mM	V_{max} / 10^{-8} M·s^{-1}	K_{cat} / s^{-1}
Free Cyt C	H_2O_2	1.76±0.06	5.06±0.09	0.66±0.01
Cyt C@BS-NPs	H_2O_2	0.89±0.04	0.61±0.05	0.08±0.01
Cyt C + 30% PEG_{4000}	H_2O_2	6.31±2.36	7.79±1.82	1.01±0.24

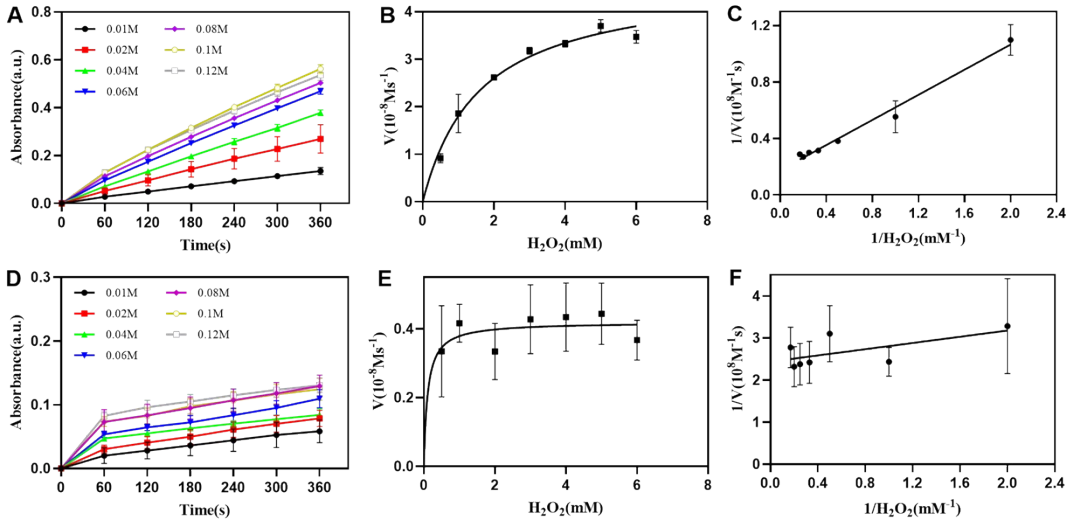

Fig. S4 Thermal stability of HRP protein. (A, D) Time-dependent absorbance changes caused by the oxTMB produced from TMB colorimetric assay with a series of concentrations of H_2O_2 in the free solution and in the confined HRP@BS-NPs. (B, E) Steady-state kinetic assay for HRP-catalyzed TMB-H_2O_2 reactions performed under different conditions: B) free solution, E) confined HRP@BS-NPs, and (C, F) are their corresponding double-reciprocal plots, respectively.

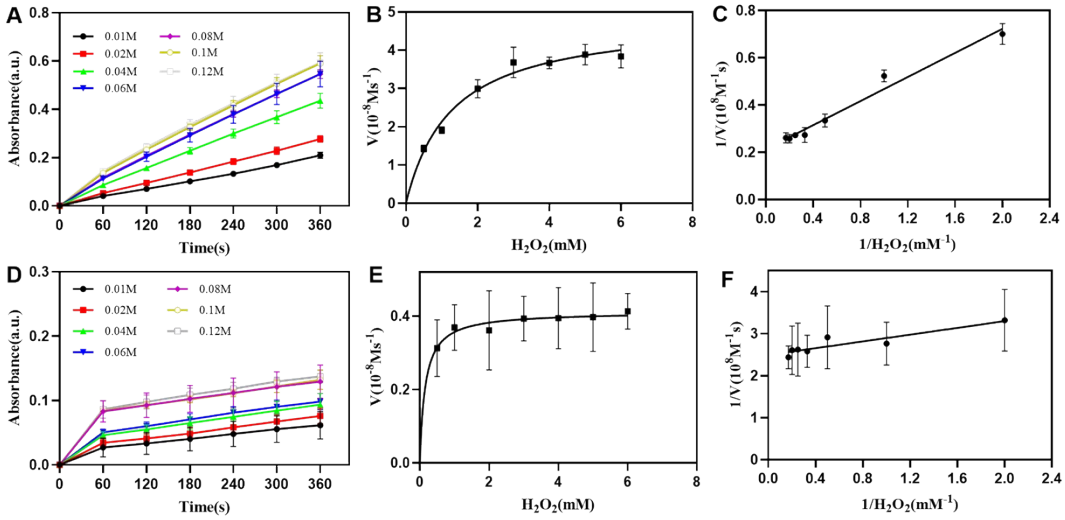

Fig. S5 Storage stability investigation of HRP protein under different conditions after a week. (A, D) Time-dependent absorbance changes caused by the oxTMB produced from TMB colorimetric assay with a series of concentrations of H_2O_2 in the free solution and in the confined HRP@BS-NPs. (B, E) Steady-state kinetic assay for HRP-catalyzed TMB-H_2O_2 reactions performed under different conditions: B) free solution, E) confined HRP@BS-NPs, and (C, F) are their corresponding double-reciprocal plots, respectively.

Table S2. Thermal stability and storage stability of HRP in the free solution.

Free HRP	**Substrate**	**K_m / mM**	**V_{max} / 10^{-8} M·s^{-1}**	**K_{cat} / 10^3 s^{-1}**	**Relative activity (%)**
HRP activity	H_2O_2	2.39±0.03	10.10±0.21	7.11±0.15	100±2.11
Thermal stability	H_2O_2	1.65±0.36	4.73±0.36	3.33±0.25	46.84±3.52
Storage stability	H_2O_2	1.28±0.23	4.87±0.43	3.43±0.30	48.24±2.00

Table S3. Thermal stability and storage stability of HRP in the confined HRP@BS-NPs.

HRP@BS-NPs	**Substrate**	**K_m / mM**	**V_{max} / 10^{-8} M·s^{-1}**	**K_{cat} / 10^3 s^{-1}**	**Relative activity (%)**
HRP activity	H_2O_2	0.25±0.07	0.50±0.01	0.35±0.01	100±2.86
Thermal stability	H_2O_2	0.30±0.02	0.45±0.06	0.32±0.04	91.43±11.43
Storage stability	H_2O_2	0.23±0.02	0.42±0.04	0.29±0.03	82.86±8.57

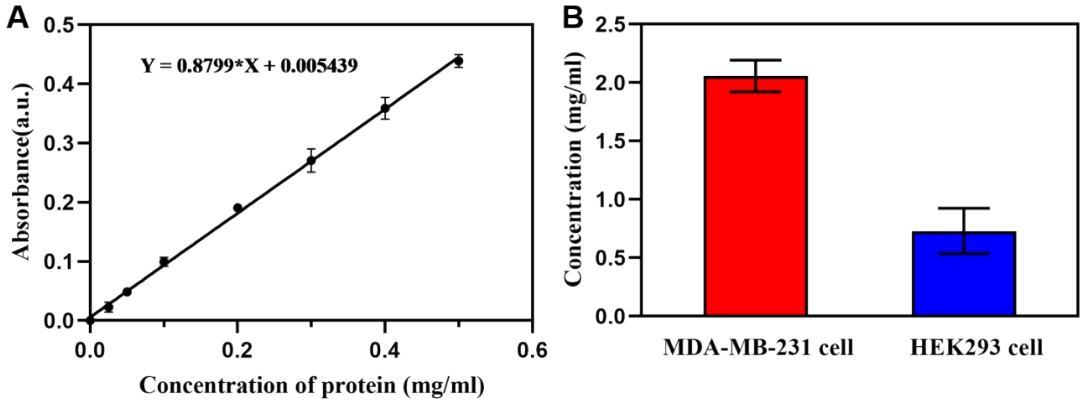

Fig. S6 Determination of the total protein content in cell extracts of different cell lines by BCA assay. (A) Linear plot of microplate reader absorbance value at 562 nm *versus* the different concentrations of BSA standard solutions. (B) Determination of the total protein content in MDA-MB-231 cancer cell extracts and HEK293 normal cell extracts by BCA assay.