Supporting Information

Platinum Nanoparticle-Based Microreactors as Support for Neuroblastoma Cells

Ana Armada-Moreira[§,†,‡], Essi Taipaleenmäki[§], Marie Baekgaard-Laursen[§], Philipp Sebastian Schattling[§], Ana M. Sebastião[†,‡], Sandra H. Vaz[†,‡], Brigitte Städler[§,].*

[§]Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus, Denmark.
[†]Instituto de Farmacologia e Neurociências, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal.
[‡]Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal.

*E-mail: bstadler@inano.au.dk

Raw fluorescence intensity data for Pt-NP-catalyzed H_2O_2 degradation.

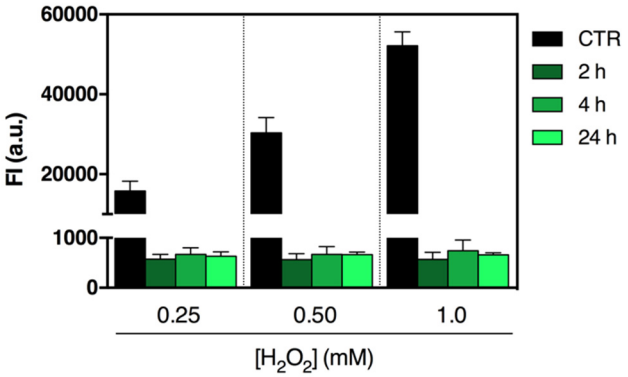

Figure S1. Pt-NP as enzyme mimics for H_2O_2 degradation. Pt-NP were incubated with different concentrations of H_2O_2 (0.25 mM, 0.50 mM, and 1.0 mM) and the remaining amount of H_2O_2 in solution was measured after 2, 4, and 24 h. Only a baseline fluorescent signal was measured after the incubation time in all cases. N=3. Data are represented as mean ± SD.

Film assembly on planar surface.

Methods. Quartz crystal microbalance with dissipation monitoring (QCM-D) measurements (Q-Sense E4, Sweden) were used to analyze the adsorption behavior of Pt-NP-containing polymer films. Silica-coated crystals (QSX300, Q-Sense) were cleaned in a 2 % (w/w) SDS solution overnight, rinsed with ultrapure water and dried with nitrogen, exposed to UV/ozone for 30 min, and pre-coated with PDA (2.5 mg mL^{-1} in TRIS buffer, 30 min). Then the crystals were dried and mounted into the chambers of the QCM-D instrument. The frequency changes (∆f) and dissipation changes (∆D) were monitored at 20 ± 0.02 °C. After a stable baseline was obtained in HEPES buffer, a PLL solution (1 mg mL^{-1} in HEPES buffer) was introduced into the chambers and let to adsorb. When the surface was saturated, the chambers were rinsed with HEPES buffer. The pre-coated PLL crystals were exposed to a Pt-NP solution until the surface was saturated and the chambers were rinsed with HEPES buffer. A layer of PLL (1 mg mL^{-1} in HEPES buffer) was adsorbed onto these pre-coated crystals prior to the exposure to either PMA (4 mg mL^{-1} in HEPES buffer) or PDA (2.5 mg mL^{-1} in TRIS buffer, 1 h, flow rate 56 µL min^{-1}). Normalized frequencies using the third harmonics are presented.

Results. The detected changes in frequency change (∆f, Figure S2A) and dissipation change (∆D, Figure S2B) of the PDA pre-coated QCM-D crystals confirmed that the film assembly via the LbL technique was successful.

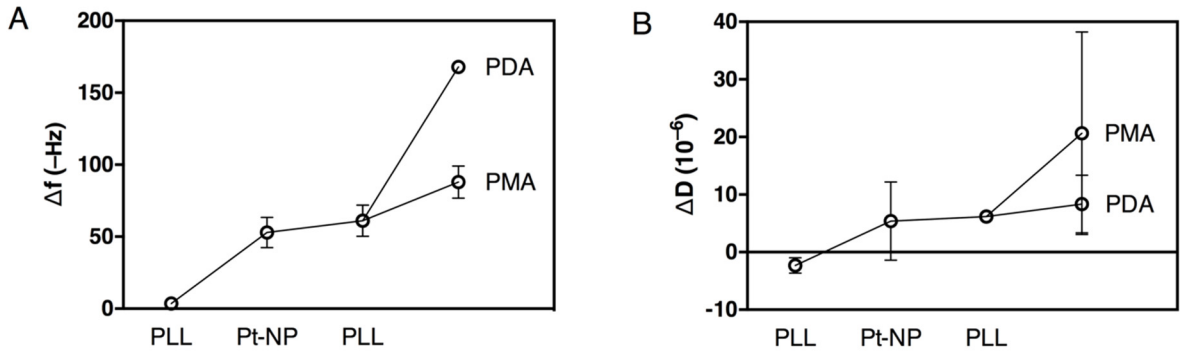

Figure S2. Changes in frequency (Δf, **A**) and dissipation (∆D, **B**) of a silica/PDA pre-coated QCM-D crystal upon deposition of PLL, Pt-NP, and PLL. Finally, either PMA or PDA were used as terminating layers.

Effects of different terminating layers on microreactor H_2O_2 scavenging properties.

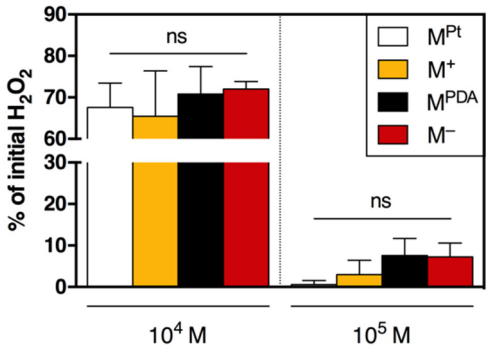

Figure S3. Pt-NP-catalyzed conversion of H_2O_2 by microreactors (M) is not affected by the terminating layer, for both 10^4 or 10^5 microreactors incubated with 1.0 mM H_2O_2 for 24 h. $^{ns}p > 0.05$ for comparison between the different microreactors. The initial concentration of H_2O_2 was set to 100% and all values were normalized to it. Data are represented as mean ± SD.

Detection of H_2O_2 consumption by microreactors in cell medium.

Methods. Cell medium was exposed to 1.0 mM H_2O_2 and 10^4 or 10^5 microreactors (M^i or M^{PDA}) for 24 h at 37 ºC. At this point, samples were centrifuged (1 min, 9 000 rpm), in order to separate the microreactors from the solution, and the supernatant was incubated with 0.16 mM o-dianisidine and 2 U mL^{-1} HRP for 30 min at room temperature. The absorbance of the solutions was read at 500 nm. To ascertain the linearity of this H_2O_2 detection assay, different concentrations of H_2O_2 (0.25 mM, 0.50 mM, and 1.0 mM) were added to cell medium in the absence of microreactors and the same protocol was performed.

Results. With this detection method, it was possible to linearly detect the concentration of H_2O_2 in cell medium for all concentrations tested (Figure S4A). Furthermore, when the microreactors were incubated with H_2O_2 in cell medium, it was possible to confirm the catalytic activity of M^{PDA}, whose actions lead to a considerable decrease of the initial H_2O_2 concentration (Figure S4B). On the other hand, no consumption of H_2O_2 was detected when H_2O_2-containing cell medium was mixed with M^i. These data corroborate that M^{PDA} maintain their catalytic activity in cell medium, thus being able to ameliorate cell viability in the presence of H_2O_2.

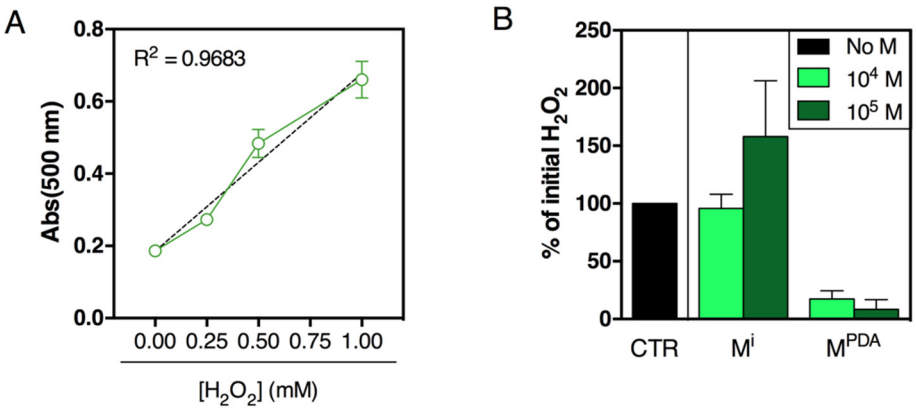

Figure S4. H_2O_2 degradation by M^{PDA} in cell medium. **(A)** Cell medium was exposed to different concentrations of H_2O_2 (0.25 mM, 0.50 mM, and 1.0 mM) and its concentration was measured, to ensure a linear response within this range of concentrations. A linear regression applied to the values obtained led to R^2 = 0.9683. N=3. Data are represented as mean ± SD. **(B)** 10^4 or 10^5 microreactors (either M^i or M^{PDA}) were incubated with 1.0 mM H_2O_2 for 24 h in cell medium. At that point, the remaining concentration of H_2O_2 in solution was measured. The value obtained for H_2O_2 in cell medium without microreactors was set to 100% and other values normalized to it. N=2. Data are represented as mean ± SD.

Cell viability: comparison between different microreactor terminating layers and different microreactor to cell ratios.

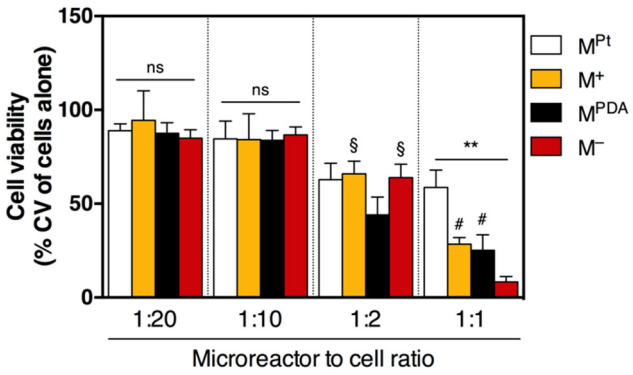

Figure S5. Effects of the terminating layers of the microreactors in cell proliferation. At lower microreactor to cell ratios, the decrease in the number of cells is independent of the terminating layer of the microreactors. $^{ns}p > 0.05$ for comparison between different microreactors. $^{**}p < 0.01$ for comparison with M^{Pt}. $^{\S}p < 0.05$ for comparison with M^{PDA}. $^{\#}p < 0.05$ for comparison with M^{-}. Cell viability for cells not exposed to microreactors was set to 100% and all other values were normalized to it. Data are represented as mean ± SD.

Cell viability in the presence of active and inactive microreactors at a one microreactor to ten cells ratio.

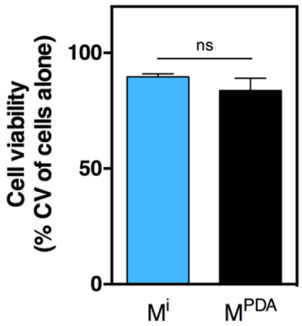

Figure S6. Cell viability of undifferentiated SH-SY5Y cells incubated with M^{i} or M^{PDA} at a ratio of one microreactor per ten cells for 24 h, normalized to the cell viability of cells not exposed to microreactors (100%). No significant differences can be observed between the two tested microreactors. $^{ns}p > 0.05$. Data represented as mean ± SD.

Dose-response curves for neuroblastoma cells in the presence of H_2O_2 or NH_4^+.

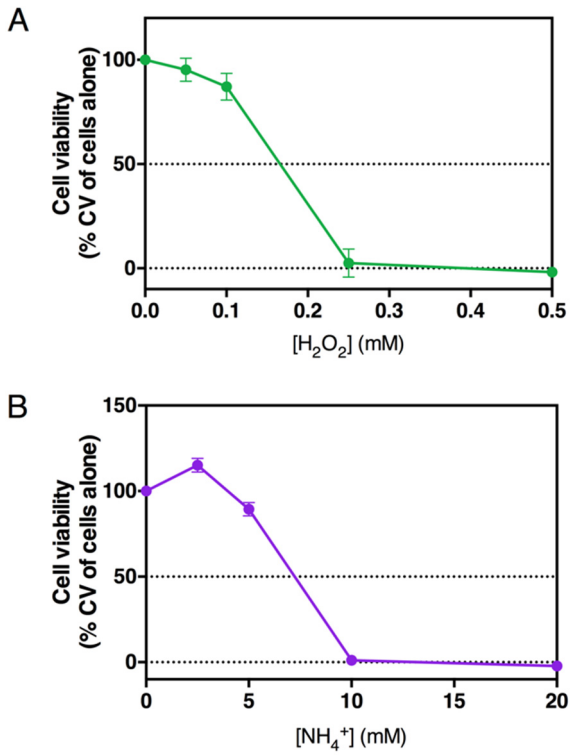

Figure S7. Cell viability curves of undifferentiated SH-SY5Y cells exposed to different concentrations of H_2O_2 or NH_4^+. Cells were exposed to different concentrations of H_2O_2 **(A)** and NH_4^+ **(B)** for 24 h prior to the cell viability measurements. A: N=3, B: N=4. The cell viability of cells not exposed to any toxic compound was set to 100% and all other values were normalized to it. Data are represented as mean ± SD.

Calculation of average activity per microreactor in solution.

Both H_2O_2 and NH_4^+ were detected using fluorescence intensity methods that provide a linear response for the range of concentrations used. Also, for both assays, a positive control was measured, using a solution with the initial concentration of H_2O_2 or NH_4^+ and no microreactors (no enzymatic consumption of the substrates).

For the H_2O_2 detection assay, the 100% of the positive control corresponds to 1.0 mM H_2O_2 (the initial concentration added to each condition), in a reaction volume of 200 µL. The percentage of H_2O_2 measured after 24 h for each condition corresponds to the amount of H_2O_2 not consumed. The results obtained using 10^4 microreactors were used for the determination of the activity per microreactor. For the 10^5 microreactors, the measured values were almost negligible, which could mean that all H_2O_2 had been already consumed over the course of the 24 h incubation. Taking the 10^4 M^{Pt} condition as an example:

$$[H_2O_2]_{initial} = 1.0\ mM\ (100\%) \qquad n(H_2O_2)_{initial} = 200\ nmol$$

$$[H_2O_2]_{final} = 675.7\ \mu M\ (67.57\%) \qquad n(H_2O_2)_{final} = 135.14\ nmol$$

$$v_{reaction} = 200\ \mu L$$

$$n(H_2O_2)_{consumed} = n(H_2O_2)_{initial} - n(H_2O_2)_{final} = 200 - 135.14 = 64.86\ nmol$$

Considering there were 10^4 microreactors in solution:

$$n(H_2O_2)_{consumed}\ microreactor^{-1} = 64.86 / 10000 = 6.49 \times 10^{-3}\ nmol$$

This value represents the amount of H_2O_2 one microreactor consumed over 24 h. To obtain the activity of one microreactor per hour:

$$n(H_2O_2)_{consumed}\ h^{-1}\ microreactor^{-1} = (6.49 \times 10^{-3}) / 24 = 2.70 \times 10^{-4}\ nmol\ h^{-1}$$

Since there were no significant differences in activity between the differently coated microreactors, it is possible to repeat this process for all tested microreactors and average their activity:

Table S1. Summary of the steps for the determination of the average activity per microreactor towards H_2O_2.

Microreactor	*% H_2O_2*	*nmol$(H_2O_2)_{final}$*	*nmol$(H_2O_2)_{consumed}$*	*nmol$(H_2O_2)_{consumed}$ microreactor^{-1}*	*nmol$(H_2O_2)_{consumed}$ h^{-1} microreactor^{-1}*
M^{Pt}	67.57	135.14	64.86	6.49×10^{-3}	2.70×10^{-4}
M^+	65.44	130.88	69.12	6.91×10^{-3}	2.88×10^{-4}
M^{PDA}	70.81	141.62	58.38	5.84×10^{-3}	2.43×10^{-4}
M^-	71.98	143.96	56.04	5.60×10^{-3}	2.34×10^{-4}

Which leads to a final average activity per microreactor of:

$$A_{average} = (2.59 \pm 0.249) \times 10^{-4}\ nmol(H_2O_2)\ h^{-1}$$

The same approach was applied to the NH_4^+ detection assay to determine the average activity per microreactor. In this case, 10^6 microreactors were incubated with 1.0 mM NH_4^+ during 24 h, in a reaction volume of 1 mL. The 100% of the positive control corresponds to 1.0 mM (and, thus, 1000 nmol) NH_4^+.

Table S2. Summary of the steps for the determination of the average activity per microreactor towards NH_4^+.

Microreactor	*% NH_4^+*	*nmol$(NH_4^+)_{final}$*	*nmol$(NH_4^+)_{consumed}$*	*nmol$(NH_4^+)_{consumed}$ microreactor^{-1}*	*nmol$(NH_4^+)_{consumed}$ h^{-1} microreactor^{-1}*
M^{Pt}	95.61	956.1	43.9	4.39×10^{-5}	1.83×10^{-6}
M^{+}	96.37	963.7	36.3	3.63×10^{-5}	1.51×10^{-6}
M^{PDA}	94.67	946.7	53.3	5.33×10^{-5}	2.22×10^{-6}
M^{-}	95.68	956.8	43.2	4.32×10^{-5}	1.80×10^{-6}

Since no significant differences between differently coated microreactors were found, averaging all determined activity values leads to a final average activity per microreactor of:

$$A_{average} = (1.84 \pm 0.291) \times 10^{-6}\ nmol(NH_4^+)\ h^{-1}$$