Supporting Information

1. Materials:

Dimyristoylphospatidylcholine (DMPC), cholesterol, dihexadecyl phosphate, horseradish peroxidase (HRP), firefly luciferase (LUC), luminol, hydrogen peroxide, D-luciferin, adenosine triphosphate (ATP), polyclonal anti-peroxidase, tetramethyl orthosilicate (TMOS) were all purchased from Sigma-Aldrich. Green fluorescent protein (GFP) was purchased from BD Biosciences. All chemicals were used as received.

2. Preparation of Phospholipid Stock Solution:

A 5:4:1 molar ratio phospholipid stock solution was prepared from DMPC, cholesterol, and dihexadecyl phosphate in chloroform. In brief, 1.670 g DMPC, 773 mg cholesterol, and 273 mg dihexadecyl phosphate were all dissolved in 10 ml of chloroform to produce a lipid cocktail of 250 mM DMPC, 200 mM cholesterol, and 50 mM dihexadecyl phosphate. This solution was stored in a sealed round bottom flask and kept inside a refrigerator until used.

3. Preparation of Enzyme-Doped Liposomes:

To prepare enzyme-doped liposomes, the chloroform in a 14 µl phospholipid stock solution was allowed to evaporate under a gentle purge of nitrogen gas in a microtube to leave behind a thin lipid film. The film was then immediately reconstituted in 28 µl dry isopropanol with vigorous mixing. Next, 500 µl 9.74×10^{-5} M HRP (10 mM phosphate buffer, pH 7.0) was added to the reconstituted phospholipid mixture and the resultant solution was then vigorously mixed for 2 minutes to form HRP-doped liposomes. To prepare GFP-doped liposomes, 500 µl 6.70×10^{-6} M GFP (10 mM TE, pH 8.0) was used instead. For LUC-doped liposomes, 50 µl of a reaction buffer (25 mM Tricine buffer, pH 7.8, 5 mM $MgSO_4$, 0.1 mM EDTA, 0.1 mM sodium azide) that contained 3.2 g/l LUC was used. Since LUC denatures easily at elevated temperatures, the LUC solution was kept in an ice bath for 1 minute prior to vigorous mixing with the lipid mixture for 30 seconds. This cooling and mixing procedure was repeated for 4 times to bring the total mixing time to 2 minutes. Right after the enzyme-doped liposomes were formed, they were made to pass back and forth 15 times through a 200 nm pore size polycarbonate membrane installed inside a mini-extrusion device (Avanti Polar Lipids) to reduce the size distribution to center around 200 nm. Enzyme-doped liposome and free enzyme were then separated once with a Sephadex G50 column before used. It is noted that using the Sephadex G50 column, the separation between enzyme-doped liposomes and free enzymes was not complete. All enzyme-doped liposomes were stored inside a refrigerator until used.

4. Removal of Free HRP from HRP-doped Liposomes for ABTS Assays:

Unlike chemiluminescence assay where free HRP was effectively inactivated by the sol-gel formation process (please refer to section 8), all free HRP has to be removed prior to an ABTS assay. This was accomplished by adding 100 µl anti-peroxidase stock to a HRP-doped liposome solution and passed the resultant solution mixture through a Protein A column to remove all free HRP from the solution. The purified HRP-doped liposomes were then stored inside a refrigerator until used.

5. HRP-Doped Liposomes in Silica Hydrogels for Chemiluminescence Assay:

A mixture of 562.5 µl TMOS, 120 µl distilled water and 11.3 µl 0.01M HCl was sonicated for 20 min in an ice bath to obtain a liquid sol first. Next a reaction cocktail that contained 64 µl HRP-doped liposomes, 144 µl distilled water, 240 µl 0.01M luminol, and 192 µl 6 % H_2O_2 was prepared inside a microtube. After thorough mixing, 80 µl liquid sol was added to the reaction cocktail and the resultant mixture was then transferred to a plastic cuvette and sealed with parafilm. Solidification of the hydrogel usually occurs within 30 minutes. To encapsulate HRP directly in a hydrogel monolith, 64 µl of 9.74×10^{-5} M HRP (10 mM Tris-HCl, pH 7.4) was used instead of the HRP-doped liposome. After solidification, the samples were subjected to multiple 1.5 kV, 20 ms electric shocks and chemiluminescence was recorded at 450 nm while the shocks were being applied. The separation between the cathode and anode was set to 1 cm to provide an electric field strength of 1.5 kV cm^{-1} when the samples were shocked.

6. HRP-Doped Liposomes in Silica Hydrogels for ABTS Assay:

A mixture of 562.5 µl TMOS, 120 µl distilled water and 11.3 µl 0.01M HCl was sonicated for 20 min in an ice bath to obtain a liquid sol first. Five identical hydrogel samples each containing a mixture of 40 µl HRP-doped liposomes (purified by the Protein A column, see section 4), 940 µl 20 mM phosphate buffer, and 140 µl silica sol solution were then prepared inside five disposable cuvettes. After the hydrogels solidified, all five samples were subjected to 87 electric shocks of 1.5 kV, 20 ms. After the shocks, five solution mixtures of ABTS (50 µM), H_2O_2 (10 mM), and phosphate buffer (20 mM, pH 7.4) with five different compositions ((80 µl, 20 µl, 100 µl), (60 µl, 20 µl, 120 µl), (40 µl, 20 µl, 140 µl), (20 µl, 20 µl, 160 µl), (10 µl, 20 µl, 170 µl)) were added to the five samples. The activity of HRP was then measured from the 420 nm absorbance from each sample using a Shimadzu UV-Visible spectrophotometer (UV-2101 PC) after 8 minutes of reaction time.

7. LUC-Doped Liposomes in Silica Hydrogels for Bioluminescence Assay:

A mixture of 562.5 µl TMOS, 120 µl distilled water and 11.3 µl 0.01M HCl was sonicated for 20 min in an ice bath to obtain a liquid sol first. Next a reaction cocktail that contained 20 µl LUC-doped liposomes, 140 µl distilled water, 50 µl reaction buffer (25 mM Tricine buffer, pH 7.8, 5 mM $MgSO_4$, 0.1 mM EDTA, 0.1 mM sodium azide) was prepared inside a microtube. After thorough mixing, 30 µl liquid sol was added to the reaction cocktail and the resultant mixture was then transferred to a plastic cuvette and sealed with parafilm. To suppress the thermal denature of LUC, the cuvette was kept at 4º C until solidification. Solidification of the hydrogel usually occurs within 30 minutes. After solidification, the hydrogel sample was broken into smaller pieces with a spatula to facilitate substrate diffusion before they were subjected to multiple 1.5 kV, 20 ms electric shocks. Bioluminescence from the sample was monitored at 560 nm upon the addition of a substrate solution that contained 4 µl 0.1 M DTT (dithiothreitol), 20 µl 10 mM D-luciferin, and 10 µl 100 mM ATP. The separation between the cathode and anode was set to 1 cm to provide an electric field strength of 1.5 kV cm^{-1} when the sample was shocked.

8. Quenching of HRP chemiluminescence:

The quenching of HRP chemiluminescence by its native substrates was observed from a sample that contained 64 µl of 9.74×10^{-5} M HRP (10 mM Tris-HCl, pH 7.4), 144 µl distilled water, 240 µl 0.01M luminol, and 192 µl 6 % H_2O_2. The chemiluminescence intensity was measured by a Shimadzu spectrophotometer (RF-5310PC) monitoring at 450 nm emission as a function of time. The solution mixture was under constant stirring throughout the entire experiment. The figure below compares the chemiluminescence intensity of free HRP in a solution with and without the addition of 80 µl liquid sol. There is a sudden increase in chemiluminescence intensity upon the addition of the liquid sol. This is immediately followed by a rapid quenching to nearly zero chemiluminescence intensity, signifying the rapid inactivation of HRP.