

## **Additional File 1**

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Methods

Additional Text

Additional Figures

### **Methods**

**Vesicle preparation.** Fatty acids and fatty acid derivatives were obtained from Nu-chek Prep (Elysian, MN). Fluorescent dyes were obtained from Invitrogen (Carlsbad, CA). Dithiothreitol (DTT) was purchased from Sigma-Aldrich (St. Louis, MO). Oleate vesicles were prepared by resuspending a dried film of oleic acid in 0.2 M Na-bicine (Sigma-Aldrich, St. Louis, MO) containing 10 mM HPTS at pH 8.5, to a final concentration of 10 mM oleic acid [3]. The vesicle suspension was vortexed briefly and then tumbled overnight. Dilutions of vesicles were made using buffers containing fatty acids above the critical aggregate concentration (cac;  $\sim 80 \mu\text{M}$  for oleic acid), to avoid vesicle dissolution. Large ( $\sim 4 \mu\text{m}$  in diameter) monodisperse multilamellar vesicles were prepared by extrusion and large-pore dialysis as described [13]. Briefly, extrusion of polydisperse vesicles through 5- $\mu\text{m}$ -diameter pores eliminates vesicles larger than 5  $\mu\text{m}$  in diameter. Dialysis of extruded vesicles against 3- $\mu\text{m}$ -pore-size polycarbonate membranes then eliminates vesicles smaller than 3  $\mu\text{m}$  in diameter, leaving behind a roughly monodisperse population of vesicles with a mean diameter of  $\sim 4 \mu\text{m}$ .

**Vesicle imaging.** Vesicles with encapsulated fluorescent dyes were imaged using a Nikon TE2000S inverted epifluorescence microscope with extra long working distance (ELWD) and oil-immersion objective lenses (Nikon, Japan). The illumination source was a 120 W metal halide lamp (EXFO, Canada) with a  $360 \pm 20 \text{ nm}$  (UV), a  $480 \pm 20 \text{ nm}$  (for HPTS), or a  $546 \pm 5 \text{ nm}$  (for Rh-DHPE) optical filter (Chroma, Rockingham, VT). The illumination intensity was

controlled using a set of two neutral density filters on the microscope. The images and movies were recorded using a digital camera (Hamamatsu Photonics, Japan) and post-processed using Phylum Live software (Improvision, Lexington, MA). High-speed vesicle explosion movies were taken by a Phantom v7.3 digital high-speed camera (Vision Research, Wayne, NJ). All images were cropped using Photoshop CS2 (Adobe Systems, San Jose, CA), with linear adjustments of brightness and contrast.

**Estimation of temperature increase.** MATLAB R2007a Pdetool (partial differential equation toolbox) (MathWorks, Natick, MA) was used to solve the thermal conduction equation in 2D. The boundary conditions were set so that the temperature at the edge of the slide was 0 °C (arbitrarily), the heat flux at the membrane/water interface was equal to the total amount of heat being generated by the internal fluorescent dye (at steady state), and the temperature difference across the vesicle membrane (~5 nm thick) was negligible.

**Photodegradation of bicine buffer.** A microcentrifuge tube containing 50  $\mu$ l 0.2 M Na-bicine (pH 8.5), 10 mM HPTS, and 0.1 M H<sub>2</sub>O<sub>2</sub> was mounted on a microscope stage and illuminated (through a 480 $\pm$ 20 nm filter) for 20 min. A longer illumination time (20 min) was used here because 1) most of the incident light was expected to be absorbed in the first 80  $\mu$ m of the ~4 mm sample and 2) we used a lower magnification (20X) lens to produce a larger illumination area to cover the entire sample, resulting in a 10 times lower illumination intensity. An Esquire 6000 ion trap mass spectrometer (Bruker Daltonics, Billerica, MA) with EsquireControl software was used to analyze the solution before and after the illumination. A vapor pressure osmometer (Wescor, Logan, UT) was used to measure the osmolarity changes before and after the illumination. To measure the critical osmotic gradient for membrane rupture, a micropipette pulled from a thin-wall glass capillary tube (Fisher Scientific, Pittsburgh, PA) was held in place by a three-axis oil hydraulic fine micromanipulator (Narishige, Japan). The micropipette was connected to a manual microsyringe pump (World Precision Instruments, Sarasota, FL), which

was used to push vesicles into a hypotonic solution in a depression on a cell-culture glass slide (Erie, Portsmouth, NH). The vesicles were illuminated under low intensity illumination for imaging. Bicine buffers at lower concentrations ( $\Delta$  concentration of 6.7, 13.3, 20, 26.7, and 33.3 mM, or  $\Delta$  osmolarity of 10, 20, 30, 40, and 50 mOsm/L, respectively) but with the same pH (8.5) were used as hypotonic solutions. Only when diluting into hypotonic bicine buffers at  $\Delta$  osmolarity of 20 mOsm/L and above was membrane rupture observed.

**NMR measurements.**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) measurements were performed on a Varian 400 NMR spectrometer (Varian, Palo Alto, CA) at 25 °C and data analyzed using the Varian VnmrJ 2.1B software. A microcentrifuge tube containing 40  $\mu\text{l}$  1 M Na-bicine, 10 mM HPTS, and 0.5 M  $\text{H}_2\text{O}_2$  in  $\text{D}_2\text{O}$  (pH 8.5) was mounted on a microscope stage and illuminated through a  $480\pm 20$  nm filter for 20 min; this procedure was repeated 10 times to collect a 400  $\mu\text{l}$  sample. HMBC (Heteronuclear Multiple-Bond Correlation) spectra: gradient selection and adiabatic pulses contained  $2048\times 1024$  data points (256 transients/128 increments). Spectra were Fourier-transformed using a non-shifted sine-bell in both dimensions. The heteronuclear correlations were optimized for  $^1J_{\text{C,H}} = 180$  Hz and  $^nJ_{\text{C,H}} = 8$  Hz. Spectral widths were adjusted accordingly.

**Photoactivated exploding vesicles for localized drug release.** The A549 (human lung adenocarcinoma) cell line was obtained from ATCC (Manassas, VA). The cells were cultured in glass-bottom culture dishes (MatTek, Ashland, MA) using 1X F-12K Nutrient Mixture medium (Invitrogen, Carlsbad, CA) containing 10% FBS and 10  $\mu\text{g/ml}$  gentamycin. The cells were cultured for 9 days before use, with the cell culture medium changed every 3 days. POPC:oleate (4:1) vesicles with 6 mol % PE-PEG2000 (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]) containing cisplatin (*cis*-dichlorodiammine platinum(II)) or carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato) platinum) (Sigma-Aldrich, St. Louis, MO) were prepared by rehydrating a dried lipid film containing the corresponding lipids,

in a solution that contained either 7 mM cisplatin or 27 mM carboplatin (in 15 mM HPTS, 0.2 M Na-ADA, pH 7.4), to a final concentration of 40 mM lipids. The vesicles were then extruded through 5- $\mu$ m-diameter pores and dialyzed against 3- $\mu$ m-pore-size polycarbonate membranes to remove the unencapsulated cisplatin or carboplatin and vesicles smaller than 3  $\mu$ m in diameter. Aseptic 1X D-MEM/F-12 without phenol red (Invitrogen, Carlsbad, CA) containing 10  $\mu$ g/ml gentamycin was used as the wash buffer. To ensure that any unencapsulated drugs remaining after dialysis did not inhibit cell growth, the wash buffer was collected in the last round of dialysis (which contained the same concentration of unencapsulated drugs) for later use in culturing one of the control groups. A small volume (~200  $\mu$ l) of the dialysed vesicle suspension was added to cancer cells in a glass-bottom culture dish, sealed by a glass coverslip on top to prevent evaporation. Photoactivation of exploding vesicles was achieved by illuminating the glass-bottom culture dish on a microscope through an oil-immersion lens, after which the cells were cultured in the solution for 3 more days before imaging and cell counting. Cell counting was performed in disposable hemacytometers (Incyto, South Korea) after cell resuspension by trypsin. Control experiments included 1) culturing cells in the medium collected from the last round of dialysis, to show that the concentration of unencapsulated drug was not biologically significant, 2) growing cells in the presence of plain vesicles without encapsulated drugs but under intense illumination, and 3) growing cells in the presence of drug-containing vesicles but without illumination.

**Nanoparticle release from exploding vesicles.** To encapsulate nanoparticles in vesicles, oleate vesicles were prepared in a solution containing biotin-coated fluorescent nanoparticles (40 nm in diameter, 0.1% solid suspension) (Invitrogen, Carlsbad, CA), 10 mM HPTS, and 0.2 M Na-bicine (pH 8.5). The vesicles were extruded through 5- $\mu$ m-diameter pores and dialyzed against 3- $\mu$ m-pore-size polycarbonate membranes using the methods described above, to remove both the unencapsulated nanoparticles and vesicles smaller than 3  $\mu$ m in diameter. The streptavidin coating of glass coverslips was performed using methods described in the literature [26]. A

simple PDMS (polydimethylsiloxane) microfluidic channel with an inlet and an outlet was designed using AutoCAD 2006 (Autodesk, San Rafael, CA) and fabricated by the Stanford University Microfluidics Foundry (Stanford, CA). The flow of nanoparticle-encapsulating vesicles was controlled by a manual microsyringe pump, and the vesicle explosions were triggered by intense illumination through a  $480\pm 20$  nm filter and a 60X oil-immersion lens.

***C. elegans* imaging.** Adult wild-type *C. elegans* were grown in a petri dish seeded with *E. coli* as the food source. A worm picker made by mounting a piece of 32-gauge platinum wire (Fisher Scientific, Pittsburgh, PA) on the tip of a Pasteur pipette was used to pick up and move the animals. A drop of melted 4% agar was placed onto a large glass coverslip until cooled. The animals were paralyzed in 10 mM sodium azide and placed on the agar, covered by another smaller coverslip. The animals were imaged by intense illumination through a  $360\pm 20$  nm (UV) filter and an oil-immersion lens.

## **Additional Text**

### **Estimation of temperature increase in a vesicle under illumination.**

Knowing the power output of the EXFO 120 W metal halide lamp at  $480\pm 20$  nm wavelength (EXFO product manual) and filter efficiency of 80%, we estimated that the irradiance in the field of view was  $2.5 \text{ W/mm}^2$ . The extinction coefficient of HPTS is  $2\times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ , the cross-section area of a vesicle with 4  $\mu\text{m}$  diameter was  $12\times 10^{-12} \text{ m}^2$ , and the illumination path through a vesicle (on average) was  $2\times 10^{-6} \text{ m}$ . According to the Beer–Lambert law, the incident light energy absorbed by a vesicle containing 10 mM HPTS was approximately  $2.5\times 10^6 \text{ W/m}^2 \times 12\times 10^{-12} \text{ m}^2 \times 10^{-2} \text{ M} \times 2\times 10^6 \text{ M}^{-1} \text{ m}^{-1} \times 2\times 10^{-6} \text{ m} = 1.2\times 10^{-6} \text{ W}$ .

The boundary conditions were set so that the temperature at the edge of the slide was  $0 \text{ }^\circ\text{C}$  (arbitrarily) and the heat flux at the membrane/water interface was  $1.2\times 10^{-6} \text{ W}$  (at steady state), and the temperature difference across the vesicle membrane ( $\sim 5$  nm thick) was negligible. Knowing that the thermal conductivity of water is  $0.6 \text{ W K}^{-1} \text{ m}^{-1}$ , we used MATLAB R2007a Pdetool (partial differential equation toolbox) to solve the thermal conduction equation  $q = -\nabla T \times k$  (where  $q$  is the heat flux,  $\nabla T$  is the local temperature gradient, and  $k$  is the thermal conductivity) in 2D and estimated that the maximum temperature increase in a vesicle was  $< 0.1 \text{ }^\circ\text{C}$ .

### **Estimation of photon absorption and ROS production.**

We know that the incident light energy absorbed by a vesicle containing 10 mM HPTS was  $\sim 1.2\times 10^{-6} \text{ W}$ . According to the Planck–Einstein equation  $E = hc/\lambda$  (where  $h$  is the Planck's constant,  $c$  is the speed of light, and  $\lambda$  is the light wavelength), the energy of each photon at  $\lambda = 480 \text{ nm}$  is  $4.1\times 10^{-19} \text{ J}$ . Thus in a period of 0.5 sec, assuming that the fluorescent dye properties did not change (no photobleaching), the number of photons absorbed by each vesicle was

approximately  $0.6 \times 10^{-6} \text{ J} / 4.1 \times 10^{-19} \text{ J} = 1.5 \times 10^{12}$  photons. Based on a vesicle volume of  $3.4 \times 10^{-15} \text{ L}$  and dye concentration of 10 mM, the number of HPTS molecules in a vesicle was approximately  $2 \times 10^8$ . Thus each HPTS molecule absorbed  $\sim 7.5 \times 10^3$  photons in 0.5 sec. Knowing that the ROS quantum yield for most fluorescent dyes is  $\sim 50\%$  [14], we estimated that each HPTS molecule could produce  $\sim 3.8 \times 10^3$  ROS, corresponding to  $\sim 40 \text{ M}$  ROS, in 0.5 sec (the actual value must be less considering the photobleaching of the dye molecules and availability of oxygen (solubility in water  $\sim 0.25 \text{ mM}$ ), even though oxygen molecules are permeable to the vesicle membrane). On the other hand, to reach the critical osmotic gradient for vesicle rupture at  $\sim 20 \text{ mOsm/L}$ , only 10 mM bicine molecules need to be oxidized, requiring  $\sim 40 \text{ mM}$  ROS, or 4 hydroxyl radicals from each HPTS molecule (based on the mechanism proposed in Figure S5). Therefore, we conclude that the calculated ROS production rate from the HPTS molecules in a vesicle under intense illumination should be sufficient to raise the internal osmotic pressure of a vesicle to cause membrane rupture.

### **Young–Laplace equation.**

Based on the Young–Laplace equation  $\Delta p = 2\gamma / r$  (where  $\Delta p$  is the cross-membrane pressure gradient,  $\gamma$  is the membrane surface tension, and  $r$  is the vesicle radius) for a given cross-membrane osmotic gradient, the surface tension is proportional to the vesicle radius. Thus, larger vesicles are subject to greater surface tension and are expected to explode more rapidly than smaller ones under the same illumination intensity. We estimated the rupture tension for the oleate membrane by dilution of vesicles into a series of hypotonic solutions. Vesicle rupture was observed only after dilution into buffer with a  $\Delta$  osmolarity of 20 mOsm/L or greater. However, accounting for vesicle swelling of  $\sim 5\%$  from a relaxed to a swollen spherical shape [20, 21], we estimate the final trans-membrane osmolarity gradient was  $\sim 5 \text{ mOsm/L}$ . For a vesicle radius of 2  $\mu\text{m}$ , this correspond to a rupture tension of approximately 12 dyn/cm.

## A potential drug delivery system

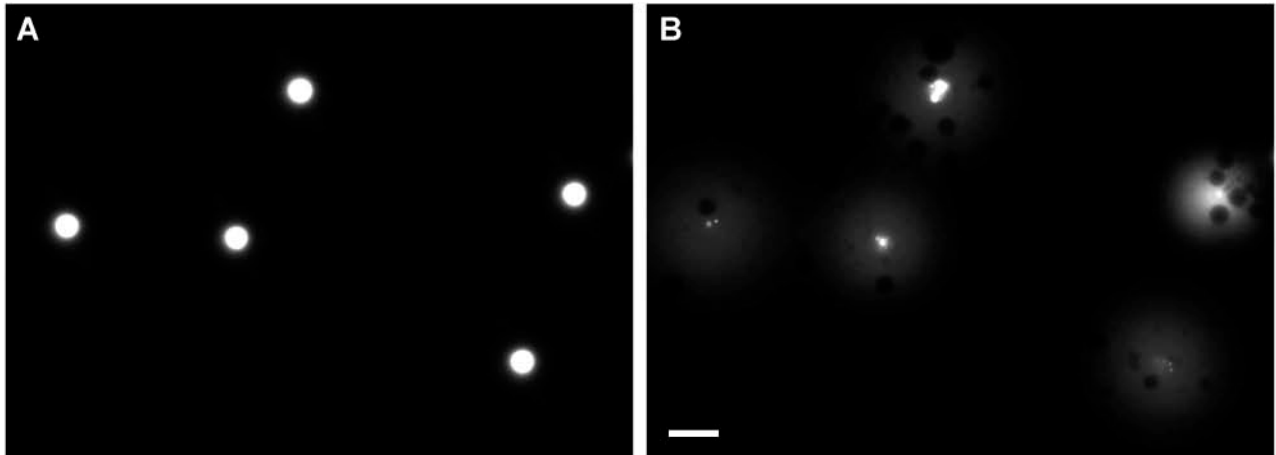
As an initial step towards translating the exploding vesicle system into a photoactivated drug delivery system, we modified the membrane compositions and the internal buffer solute. First, since fatty acids bind to albumin in blood, pure fatty acid vesicles are not stable in the circulation. Therefore, we used POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine):oleate (4:1) vesicles instead (the addition of 20 mol % oleate to the membrane helped to reduce the rupture surface tension and increase the efficiency of vesicle membrane rupture, because pure POPC vesicles require a 2.5-fold higher osmotic gradient to rupture than oleate vesicles [20]). The vesicle membrane also contained 6 mol % PE-PEG2000 (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]), to prevent cell uptake of vesicles. Second, bicine (pKa 8.35) cannot be oxidized efficiently at pH 7.4 (possibly because its tertiary nitrogen is protected when protonated), and thus vesicles containing bicine do not explode efficiently at physiological pH. We therefore replaced bicine with ADA (*N*-(2-acetamido)iminodiacetic acid, which has a structure similar to that of bicine but a pKa of 6.9) as the internal solute, which allowed the vesicles to explode efficiently at pH 7.4.

We used an *in vitro* cell culture system to carry out a proof-of-principle test of the effectiveness of this experimental photoactivated drug delivery system. Since the oxidation of cargo drugs by ROS is a concern, we used the platinum-based chemotherapy drugs cisplatin (*cis*-dichlorodiammine platinum(II)) and carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato) platinum) as model cargo drugs. Cisplatin and carboplatin cause DNA crosslinks, leading to cancer cell apoptosis [35]. The metal-coordinated ammonia ligands of these drugs are less likely to be oxidized than the tertiary amine groups of bicine or ADA, protecting the drugs from immediate radical oxidation. Human lung adenocarcinoma (A549) cells were cultured in glass-bottom culture dishes and treated with vesicles containing either 7 mM cisplatin or 27 mM carboplatin (in 15 mM HPTS, 0.2 M Na-ADA, pH 7.4), illuminated to release the drugs (Figure

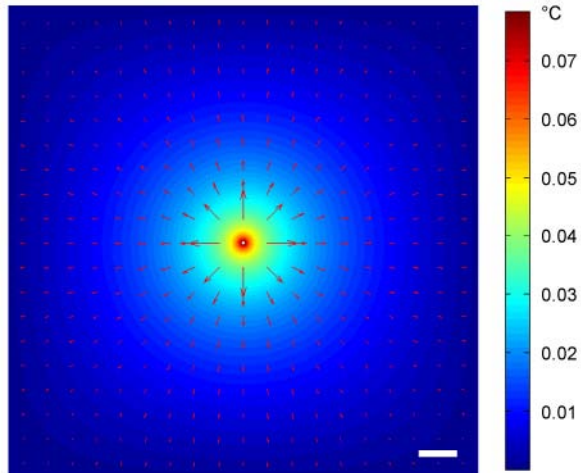
S8C, D). The treated cells have approximately 75-80% lower survival rates than those in the control groups (Figure S8E). In one control, cells cultured in the medium collected from the last round of vesicle dialysis grew normally, showing that there was no biologically significant concentration of unencapsulated drugs in the solution (Figure S8B). Similarly, cells cultured with plain vesicles without encapsulated drugs but exposed to intense illumination, or with drug-containing vesicles but without illumination, showed normal growth.

Many technical questions need to be addressed to enable practical applications of light-directed vesicle rupture. For many drug delivery applications, smaller vesicles with a diameter  $< 200$  nm are desirable [36, 37]. But controlled content release from such small vesicles would require either much higher internal osmotic pressure, or that the vesicles be composed of materials conferring much lower rupture tension. The use of far-red or infrared light to excite a photosensitizing dye with a longer excitation wavelength would be essential to achieve tissue penetration of up to a few mm [38]. Future improvements in this area may take advantage of the variety of ROS-generating photosensitizers being developed for photodynamic therapy (some of which are already in clinical use or in clinical trials) [39, 40].

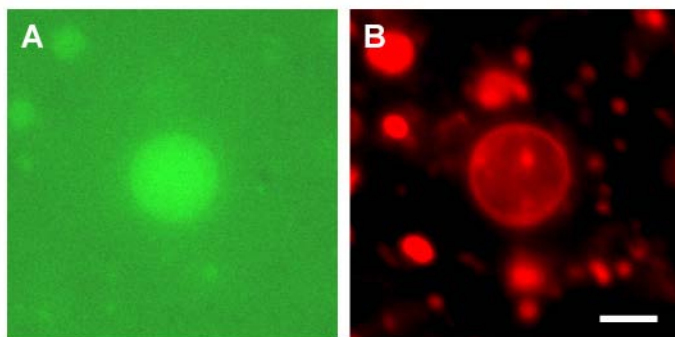
## Additional Figures



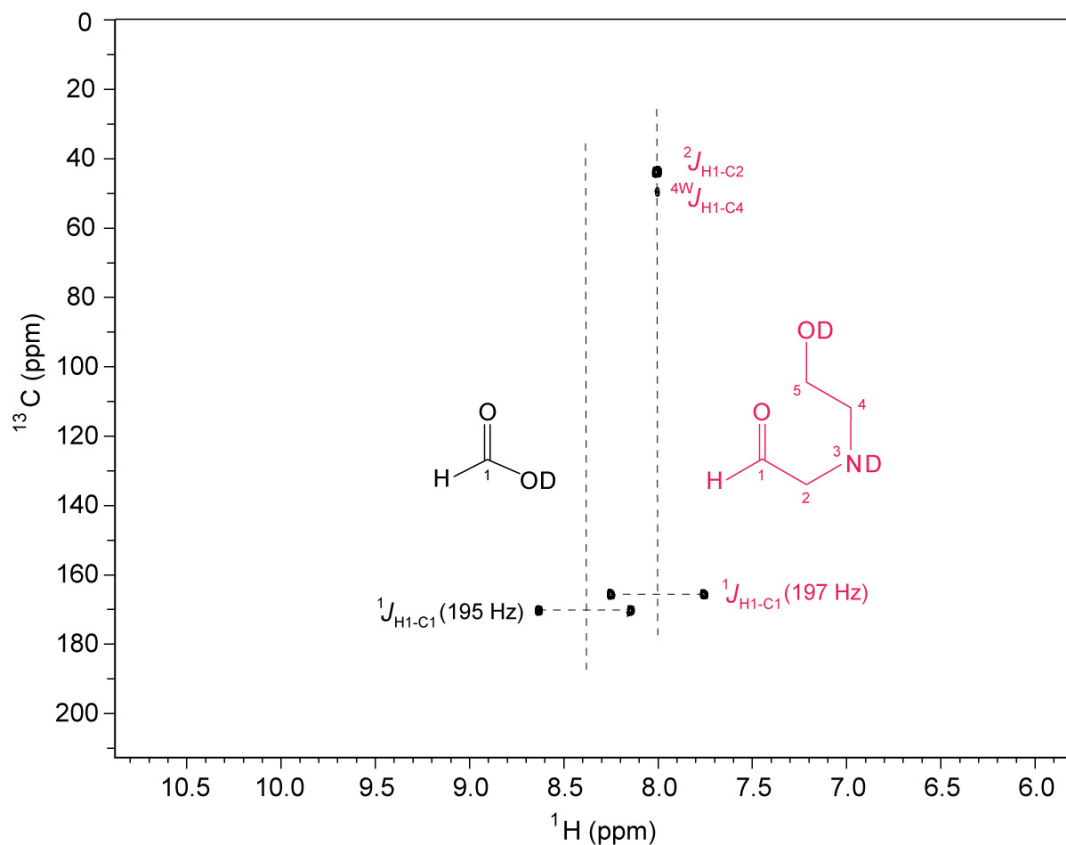
**Figure S1 | Exploding vesicles.** **A, B**, Oleate vesicles (containing 10 mM HPTS, in 0.2 M Na-bicine, pH 8.5) exploded shortly (~0.5 sec) after being exposed to intense illumination (Additional File 2). Scale bar, 10  $\mu$ m.



**Figure S2 | Estimated temperature increase in a vesicle under intense illumination.** Pseudo colours correspond to the temperature increase in water surrounding a vesicle with a constant heat flux of  $1.2 \times 10^{-6}$  W, solved in 2D and plotted by MATLAB. The maximum temperature increase immediately outside the vesicle membrane (shown as a small white circle in the middle) was estimated  $< 0.1$  °C. Heat flux vectors are shown in red arrows. Scale bar, 100  $\mu$ m.

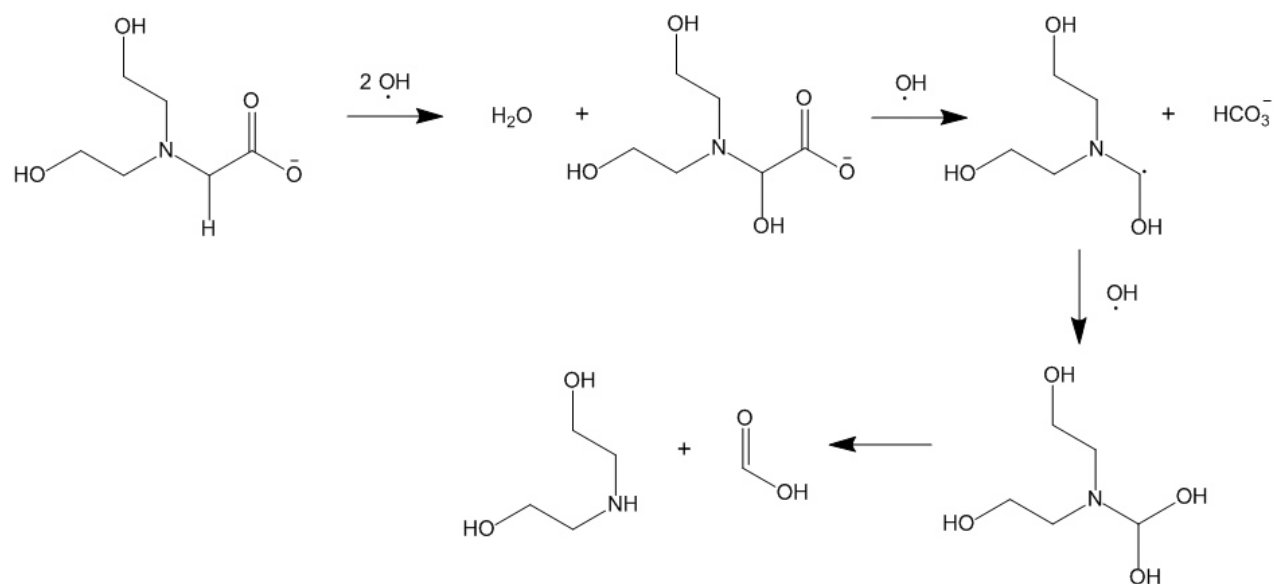


**Figure S3 | Equal concentrations of fluorescent dye inside and outside the membrane.** **A**, A large vesicle (with 10 mM HPTS inside and outside the membrane, in 0.2 M Na-bicine, pH 8.5) remained intact after intense illumination for 10 sec. **B**, The vesicle was labeled by a membrane-localized dye (Rh-DHPE). Scale bar, 5  $\mu$ m.



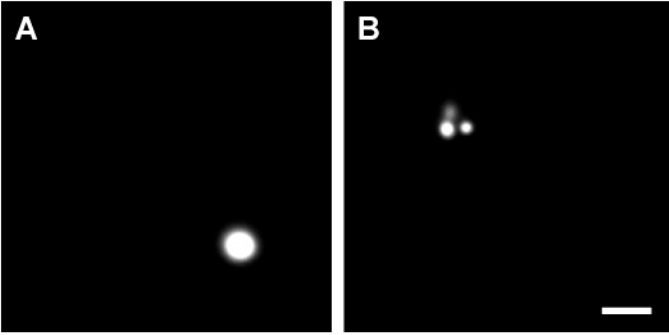
**Figure S4 | 2D gHMBC  $^1\text{H}$ - $^{13}\text{C}$  NMR analysis of bicine oxidation products in  $\text{D}_2\text{O}$ .**

The non-exchangeable proton of formic acid (left, in black) has a characteristic  $^1\text{H}$  chemical shift at 8.4 ppm and was unambiguously assigned by spiking with commercially available HPLC grade formic acid, which resulted in an increased intensity of this peak (observed with 1D  $^1\text{H}$  spectra, data not shown). In addition, this proton only had a one-bond correlation (shown as characteristic splitting of 195 Hz as leakage signal from low-pass  $J$  filter in the HMBC experiment) with the carboxyl/carbonyl carbon ( $^{13}\text{C}$  chemical shift at  $\sim 170.3$  ppm), indicating that this compound was indeed formic acid. Another detected compound is likely to be 2-(2-hydroxyethylamino)acetaldehyde (right, in red), an oxidation product of diethanolamine [17]. The cross-peaks of proton-carbon connections were assigned as labelled and numbered in the figure.

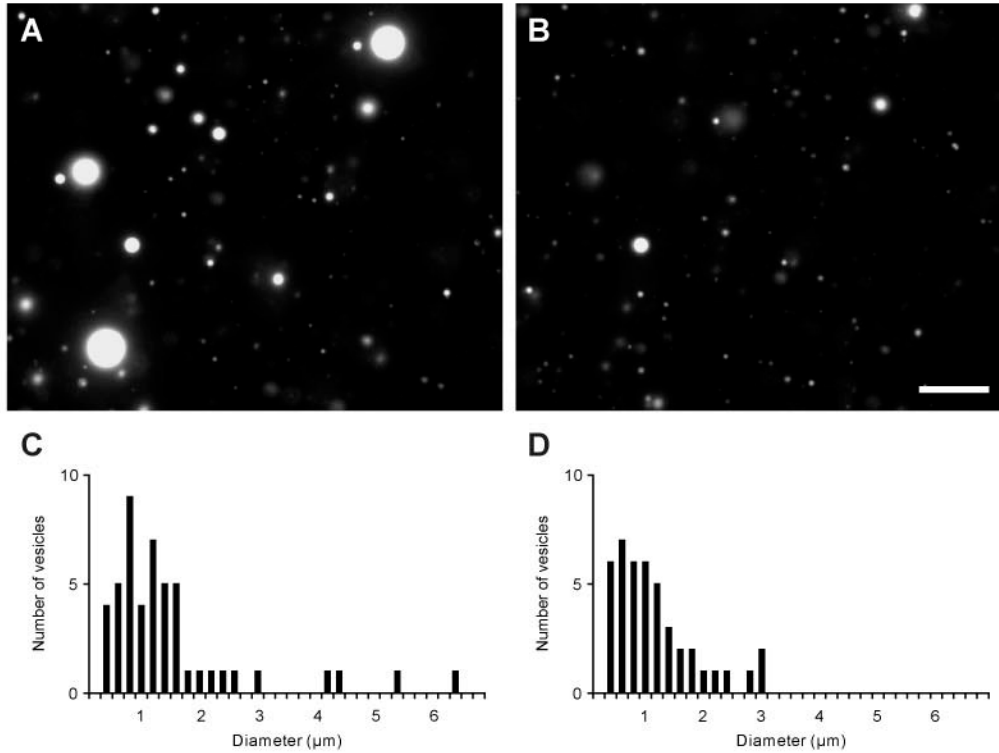


**Figure S5 | Proposed mechanism for the radical-mediated oxidation of bicine.**

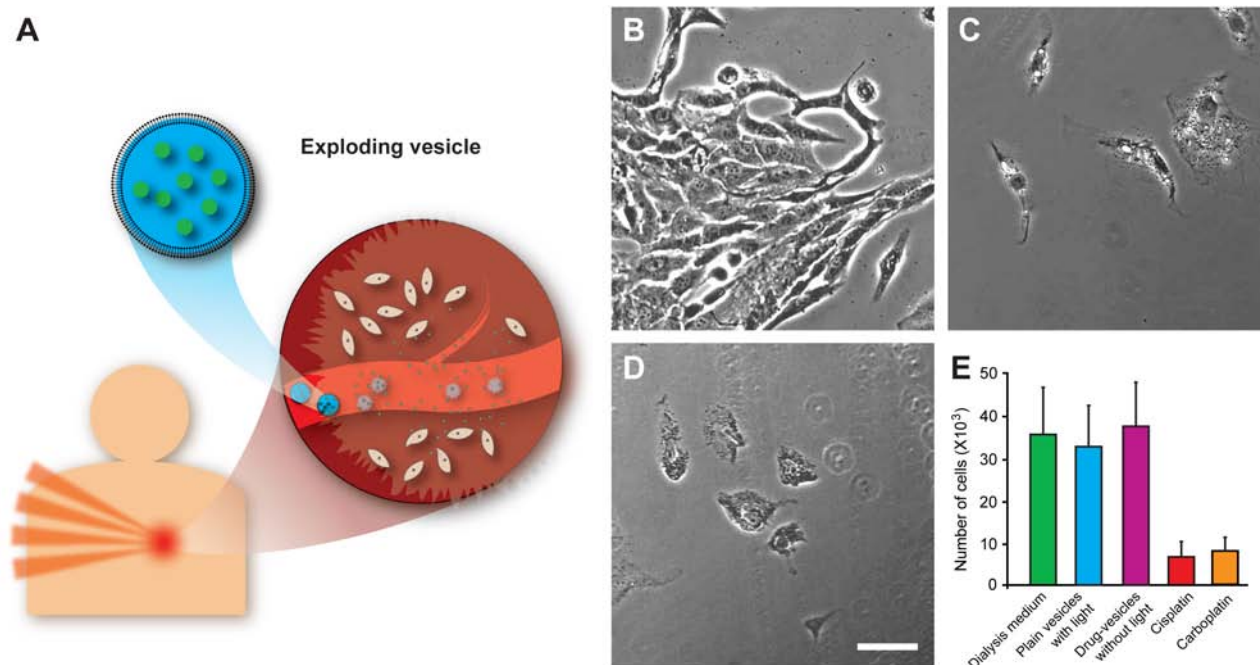
Oxidation by hydroxyl radicals produced by illumination of fluorescent dyes [41]. The proposed products include: diethanolamine, formic acid, and bicarbonate (at pH 8.5).



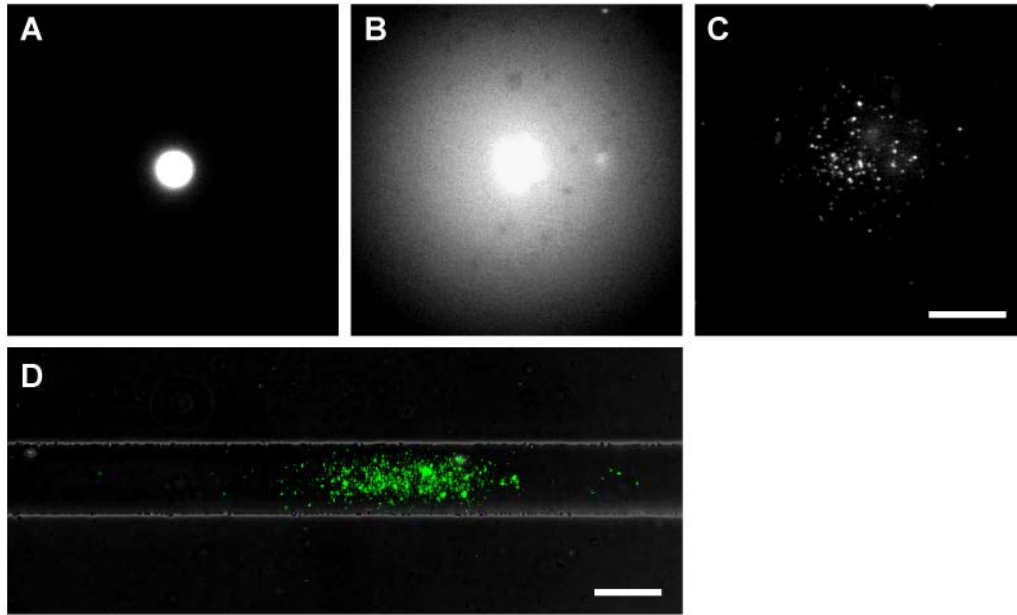
**Figure S6 | Critical osmotic gradient for membrane rupture.** **A**, Diluting a vesicle into a hypotonic solution ( $\Delta$  concentration of 13.3 mM, or  $\Delta$  osmolarity of 20 mOsm/L) through a micropipette. **B**, The outer membranes ruptured, and the smaller internal vesicles were released (under low intensity illumination for imaging) (Additional File 6). Scale bar, 5  $\mu$ m.



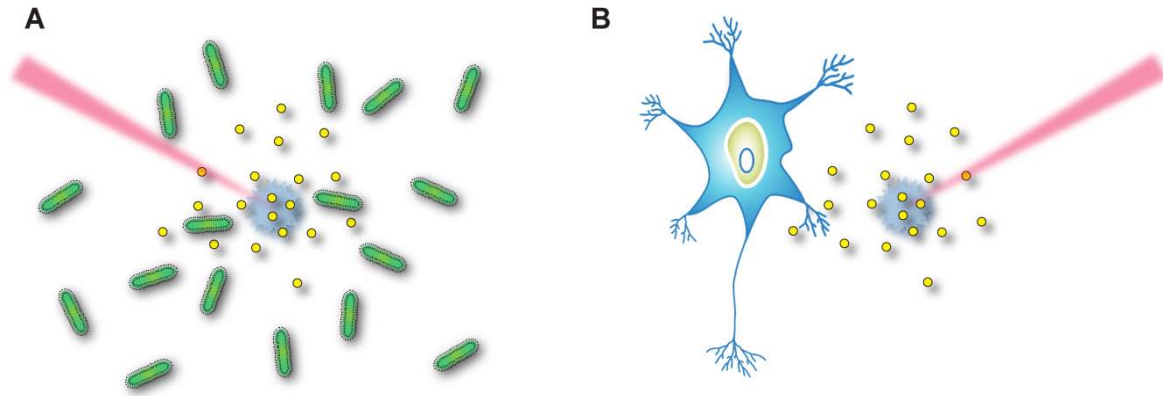
**Figure S7 | Size dependence of vesicle explosions.** **A, B**, In a population of polydisperse oleate vesicles (containing 10 mM HPTS, in 0.2 M Na-bicine, pH 8.5), under intense illumination, the larger (> 3 μm in diameter) vesicles exploded rapidly while the smaller ones remained intact (Additional File 7). Scale bar, 10 μm. **C, D**, The corresponding size distributions before and after intense illumination, shown as number of vesicles per 0.2 μm bin (vesicles between 0-0.2 μm in diameter were not counted).



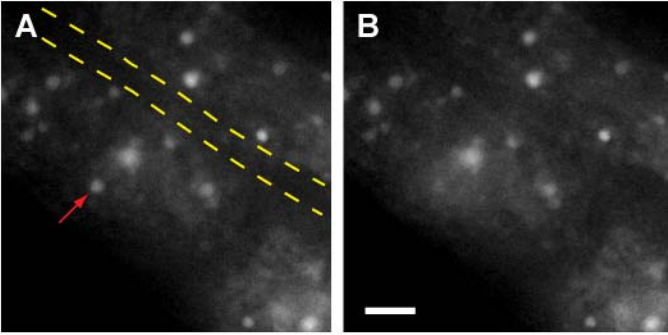
**Figure S8 | Exploding vesicles for localized drug release.** **A**, Schematic diagram for a potential strategy to localize the release of chemotherapy drugs from exploding vesicles through photoactivation. **B**, In one control group, human lung adenocarcinoma (A549) cells were cultured in the medium collected from the last round of dialysis. Cells were imaged using differential interference contrast microscopy (DIC). **C**, **D**, Cells treated by cisplatin or carboplatin released from photoactivated exploding vesicles (containing either 7 mM cisplatin or 27 mM carboplatin, in 15 mM HPTS, 0.2 M Na-ADA, pH 7.4), respectively. Scale bar, 50  $\mu$ m. **E**, Number of total viable cells from the treated groups and the control groups (cells cultured in the medium collected from the last round of (cisplatin) dialysis, cells treated with plain vesicles but under intense illumination, and cells treated with drug-containing (cisplatin) vesicles but without illumination). Error bars show s.d. ( $n = 3$ ).



**Figure S9 | Nanoparticle release from exploding vesicle.** **A, B**, An oleate vesicle (with a diameter of  $\sim 4 \mu\text{m}$ , containing 10 mM HPTS, in 0.2 M Na-bicine, pH 8.5) encapsulating biotin-coated fluorescent nanoparticles (nanoparticles were 40 nm in diameter) exploded shortly after being exposed to intense illumination, releasing a cloud of nanoparticles (Additional File 8). **C**, The nanoparticles attached to a streptavidin-coated glass surface. Scale bar for **A-C**, 10  $\mu\text{m}$ . **D**, Using exploding vesicles to localize the release of nanoparticles to a specific area in a microfluidic channel. Scale bar for **D**, 50  $\mu\text{m}$ .



**Figure S10 | Schematic diagram for other potential applications of exploding vesicles. A,** Using exploding vesicles to release signaling molecules for studying bacterial chemotaxis. **B,** Using exploding vesicles to release neurotransmitters for activating neurons.



**Figure S11 | Lysosome explosions in adult *C. elegans*.** **A, B**, Under intense UV ( $360\pm 20$  nm) illumination, autofluorescent lysosomes (red arrow) exploded, releasing the lysosomal contents (Additional File 9). The cells shown in the images were gut granule cells (yellow dashed lines show the gut lumen). Scale bar, 5  $\mu$ m.