Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Spectrally Tunable Uncaging of Biological Stimuli from Nanocapsules

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Optical set up

One-Photon. We used a 3ns (pulse width) 337nm N_2 laser (Laser Sciences, Inc., Franklin, MA) with a dye head attachment for photolysis of single nanocapsules. The dye head contained a solution of DCM (Exciton, Inc., Dayton, OH), which emitted at the desired wavelength (645nm). For epi-fluorescence imaging, a blue solid-state laser (Coherent, Inc., Santa Clara, CA) of wavelength at 488nm was employed. The emitted fluorescence was collected by a high numerical aperture objective (N.A. 1.3; 100×) with expanded transmission in the UV and the NIR range (Nikon Superfluor, Mellville, NY), passed through dichroic and band-pass filters (Chroma Tech. Corp., Rockingham, VT), and imaged onto a CCD camera (4900 Series, Cohu, San Diego, CA).

Two-Photon. We used an 80 MHz mode-locked, Tsunami Ti:Sapphire laser (Spectra Physics, Mountain View, CA) tuned to 710nm for photolysis of single nanocapsules. For epi-fluorescence imaging, a green laser (Beam of Light Technologies, Clackamas, OR) of wavelength 532nm was employed. The emitted fluorescence was collected by a high numerical aperture objective (N.A. 1.3; 100×) with expanded transmission in the UV and the NIR range (Nikon Superfluor, Mellville, NY), passed through dichroic and band-pass filters (Chroma Tech. Corp., Rockingham, VT), and imaged onto a CCD camera (4900 Series, Cohu, San Diego, CA).

Preparation of vesicles

Materials. The mini-extruder and all lipids were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). All dyes were purchased from Invitrogen, Molecular Probes (Carlsbad, CA). Carbachol was purchased from Lancaster Synthesis, Alfa Aesar (Ward Hill, MA). Bradykinin was purchased from AnaSpec, Inc. (San Jose, CA). All other materials and reagents were purchased from VWR (West Chester, PA).

Vesicle Synthesis. Lipids and membrane dyes were combined in a vial and dried under N_2 for at least 45 minutes. The samples were then further dried under vacuum for more than 90 minutes in order to ensure complete removal of the original solvent, chloroform. Amounts of encapsulated material were added with buffer to the vial, and then the entire solution was diluted to 2mM (with respect to lipid concentration) with phosphate buffered saline, pH = 7.5. This solution was then vortexed for 1 minute to create multi-lamellar vesicles before being passed through a mini extruder that contained either one of 100, 400, or 600nm pore sizes no less than 43 times. Vesicles were then subjected to 3-5 freeze-thaw cycles to maximize amount of encapsulated material. Non-encapsulated molecules were removed by passing the vesicle solution through a size-exclusion column (Sephacryl 100, Amersham Biosciences) two times. The vesicle solution was then diluted to 2μM with respect to the lipid concentration. Vesicles were always made fresh the same day the photolysis and cellular activation experiments were carried out. The best results were when vesicles were used within 24 hours of formation.

Cell culture and activation

Materials. All cells and media and supplements were purchased from ATCC (Manassas, VA). PDMS (Sylgard 184) was bought from Dow Corning Co. (Midland, MI). No. 1 Gold Seal coverslips were from Erie Scientific (Portsmouth, NH).

Biological Buffer. A biological buffer was employed during the loading of the calcium indicator dyes. The buffer contained 140mM NaCl, 4mM KCl, 1mM NaH_2PO_4, 0.9mM $MgSO_4$, 10mM HEPES, and 25mM glucose. The final pH was adjusted to 7.4.

Preparation of PDMS Wells. Simple wells of polydimethylsiloxane (PDMS) bonded to No. 1 glass coverslips were fabricated and used to contain cells for the cellular-response experiments. Formation of wells and chips of PDMS bonded to glass is a widely used technique and has been described in detail elsewhere.[1] Briefly, PDMS prepolymer and curing agent were mixed at a 10:1 ratio, respectively, and poured into a large petri dish containing clean vials that created voids about 25mm in diameter. PDMS was then cured in an oven at 75 °C for 1 hour. The PDMS was bonded and sealed to glass coverslips (cleaned in a 1:1:1 solution of water : NH_4OH : H_2O_2) by oxidizing the respective surfaces in an expanded plasma chamber purchased from Harrick Plasma (Ithaca, NY). Wells were typically 10-15mm deep and held about one mL of solution.

CHO-M1. CHO-M1 cells (CRL-1984) were grown in RPMI-1640 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were grown to 50-85% confluence in PDMS wells just prior to each experiment. For the one-photon experiments, cells were incubated in 1μM fluo3-Am-permeate in biological buffer (no calcium present) for one hour, and washed 3 times with biological buffer just prior to experiments. Then, 1-10μL of the 2μM solution of vesicles containing carbachol (as described above) was added to the cells in the wells. Vesicles typically took about 5 min to settle near the cells in sufficient numbers for the photolysis experiments. Photolysis and cellular-response experiments were performed in RMPI-1640 media. For the two-photon experiments, cells were incubated in 1.25μM rhod-2-Am-permeate in biological buffer (no calcium present) for 1 hour rather than in 1μM fluo3-Am-permeate. All other steps remain the same as in the one-photon photolysis experiments.

PC12. PC12 (CRL-1721) cells were grown in F12K media supplemented with 15% horse serum, 2.5%fetal bovine serum, 1% penicillin-streptomycin, and nerve growth factor (NGF). Cells were grown on rat-tail collagen-coated, circular (25mm diameter), glass coverslips. PDMS wells could not be used because the PC12 cells preferred to grow up along the PDMS walls instead of covering the collagen-coated surface. For the one-photon experiments, cells were incubated in 1μM fluo3-Am-permeate in biological buffer (no calcium present) for one hour, and washed 3 times with biological buffer just prior to experiments. The circular coverslips were then placed in a small chamber designed to hold our circular coverslips. The coverslip was only held in place at the edges so that the middle was exposed for contact with our 100× objective. Two o-rings held the coverslip in place and seal the chamber to contain the liquid. One mL of the supplemented F12K media was added to the chamber after the dye incubation and washing steps. Then, 1-10μL of the 2μM solution of vesicles containing bradykinin (as described above) was added to the chamber. Photolysis and cellular experiments were performed in F12K media.

References

1. D.C. Duffy, J. Cooper McDonald, O.J.A. Schueller and G.M. Whitesides, Anal. Chem. 1998, 70, 4974-4984.