17.1 Caging Groups and Their Photolysis

Overview of Caged Probes

Flash photolysis of photoactivatable or "caged" probes provides a means of controlling the release — both spatially and temporally — of biologically active products or other reagents of interest.[1–6] The chemical caging process may also confer membrane permeability on the caged ligand, as is the case for caged cAMP[7] and caged luciferin.[8] Molecular Probes' extensive selection of caged nucleotides, chelators, second messengers and neurotransmitters has tremendous potential for use with live cells.

In addition to caged versions of biologically active molecules, we prepare caged fluorescent dyes that are essentially nonfluorescent until after photolysis. These caged fluorophores have proven useful for photoactivation of fluorescence (PAF) experiments, which are analogous to fluorescence recovery after photobleaching (FRAP) experiments except that the fluorophore is activated upon illumination rather than bleached. Measuring the bright fluorescent signal of the photoactivated fluorophore against a dark background is intrinsically more sensitive than measuring a dark photobleached region against a bright field.[1,9] We have also introduced antibody and streptavidin conjugates of CMNB-caged fluorescein (Section 7.3, Section 7.6) that develop strong green fluorescence following brief illumination with UV light (Figure 7.65).

Properties of Different Caging Groups

The caging moiety (Table 17.1) is designed to *maximally* interfere with the binding or activity of the molecule. It is detached in microseconds to milliseconds by flash photolysis at ≤360 nm, resulting in a pulse of the active product. Uncaging can easily be accomplished with UV illumination in the fluorescence microscope or with a UV laser or UV flashlamp. The availability of low-cost UV lasers[10] and UV flashlamps[11] should facilitate photolysis experiments in many laboratories (Table 17.2). A high-resolution, confocal laser-scanning microscope and flash photolysis system for physiological studies has been described.[12] The effects of photolytic release are frequently monitored either with fluorescent probes that measure calcium, pH, other ions or membrane potential, or with electrophysiological techniques.

To date, most of the caged reagents described in the literature have been derivatives of *o*-nitrobenzylic compounds. The nitrobenzyl group is synthetically incorporated into the biologically active molecule by linkage to a hetero-atom (usually *O*, *S* or *N*) as an ether, thioether, ester (including phosphate or thiophosphate esters), amine or similar functional group. Both the structure of the nitrobenzylic compound and the atom to which it is attached affect the efficiency and wavelength required for uncaging. We currently use five different photolabile protecting groups in our caged probes.[2] Their properties are summarized in Table 17.1.

Table 17.1 Properties of five different caging groups.

Probe	Uncaging Rate *	Photolysis Quantum Yield *	Inertness of Photolysis By-product	Confers Water Solubility	Long-Wavelength Absorption (≥360 nm)
CNB	++++	+++++	+++++	+++++	++
NPE	+++	+++	+++	+	++
DMNB	+++	+++	++	+	+++++
DMNPE	+++	+++	+++	+	+++++
CMNB	+++	+++	+	++++	+++

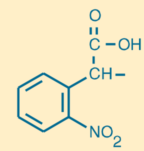
+++++ = Optimal response. + = Poor response.

* Both the structure of the nitrobenzyl moiety and the atom to which it is attached have some effect on the efficiency and wavelength required for uncaging.

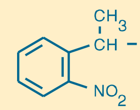
TECHNICAL NOTE

Structures of Five Different Caging Groups

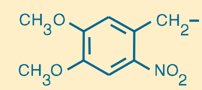
CNB =



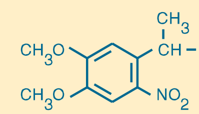
NPE =



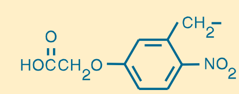
DMNB =



DMNPE =



CMNB =



TECHNICAL NOTE

Why Use Caged Probes?

Caged probes permit the rapid release of *natural* biologically active molecules or effectors, (e.g., nucleotides, Ca^{2+}, ionophores, inositol 1,4,5-triphosphate and neurotransmitters). When combined with our other probes for signal transduction, caging technology provides results that cannot be obtained by any other means, including patch clamping. It is also possible to "turn on" fluorescence and to create a hapten using caged fluorescent dyes.

Technical Assistance at Our Web Site (www.probes.com)

Molecular Probes' Web site contains the complete text of this Handbook as well as a number of other features to assist the researcher, including:

- **Product searches** by product name or catalog number
- **Color photomicrographs** that show our products in action
- **Bibliographies** for all products for which we have references
- **Keyword searches** of our entire bibliography of over 44,000 references
- **Product information sheets** for many kits and reagents
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- Probes caged with our patented α-carboxy-2-nitrobenzyl (CNB) caging group [13] generally have the most advantageous properties. These include good water solubility, very fast uncaging rates in the microsecond range, high photolysis quantum yields (from 0.2–0.4) and biologically inert photolytic by-products. Although the absorption maximum of the CNB caging group is near 260 nm, its absorption spectrum tails out to approximately 360 nm, allowing successful photolysis using light with wavelengths ≤360 nm.
- The 1-(2-nitrophenyl)ethyl (NPE) caging groups have properties similar to those of CNB and can also be photolyzed at ≤360 nm.
- As compared with CNB and NPE, the 4,5-dimethoxy-2-nitrobenzyl (DMNB) and 1-(4,5-dimethoxy-2-nitrophenyl)ethyl (DMNPE) caging groups have longer-wavelength absorption (absorption maximum ~355 nm) and therefore absorb 340–360 nm light more efficiently. However, photolysis rates and quantum yields of DMNB- and DMNPE-caged probes are generally lower than those obtained for CNB-caged probes.
- The 5-carboxymethoxy-2-nitrobenzyl (CMNB) caging group provides an absorption maximum of intermediate wavelength (absorption maximum ~310 nm), while imparting significant water solubility to the caged probe. Its photolysis rate and quantum yield are intermediate between those of CNB- and DMNB-caged probes.

Experiments utilizing probes caged with any of the above caging groups, except the CNB caging group, may require the addition of dithiothreitol (DTT, D-1532; Section 2.1). This reducing reagent prevents the potentially cytotoxic reaction between amines and the 2-nitrosobenzoyl photolytic by-products.[14]

References

1. Methods Enzymol 291, 63 (1998); **2.** Methods Enzymol 291, 30 (1998); **3.** Curr Opin Neurobiol 6, 379 (1996); **4.** Biological Applications of Photochemical Switches, Morrison H, Ed. pp. 243–305 (1993); **5.** Optical Microscopy: Emerging Methods and Applications, Herman B, Lemasters JJ, Eds. pp. 27–85 (1993); **6.** Annu Rev Physiol 55, 755 (1993); **7.** Nature 310, 74 (1984); **8.** Biotechniques 15, 848 (1993); **9.** Cell Biology: A Laboratory Handbook, 2nd Ed., Vol. 3, Celis JE, Ed. pp. 127–135 (1998); **10.** J Neurosci Methods 66, 47 (1996); **11.** Pflugers Arch 411, 200 (1988); **12.** Cell Calcium 21, 441 (1997); **13.** US 5,635,608; **14.** Annu Rev Biophys Biophys Chem 18, 239 (1989).

Table 17.2 Suppliers of instrumentation for photolysis of caged compounds.

Company	Location	Web Site
Cairn Research Ltd.	Faversham, UK	www.cairnweb.com
Intracellular Imaging, Inc.	Cincinnati, OH, USA	www.intracellular.com
Fryer Company, Inc.	Huntley, IL, USA	www.fryerco.com/prairie
Hi-Tech Scientific	Salisbury, UK	www.hi-techsci.co.uk
Photonic Instruments, Inc.	Arlington Heights, IL, USA	www.photonic-instruments.com
Prairie Technologies, LLC	Middleton, WI, USA	www.prairie-technologies.com
Rapp OptoElectronic	Hamburg, Germany	www.rapp-opto.com
T.I.L.L. Photonics	Martinsried, Germany	www.till-photonics.com

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