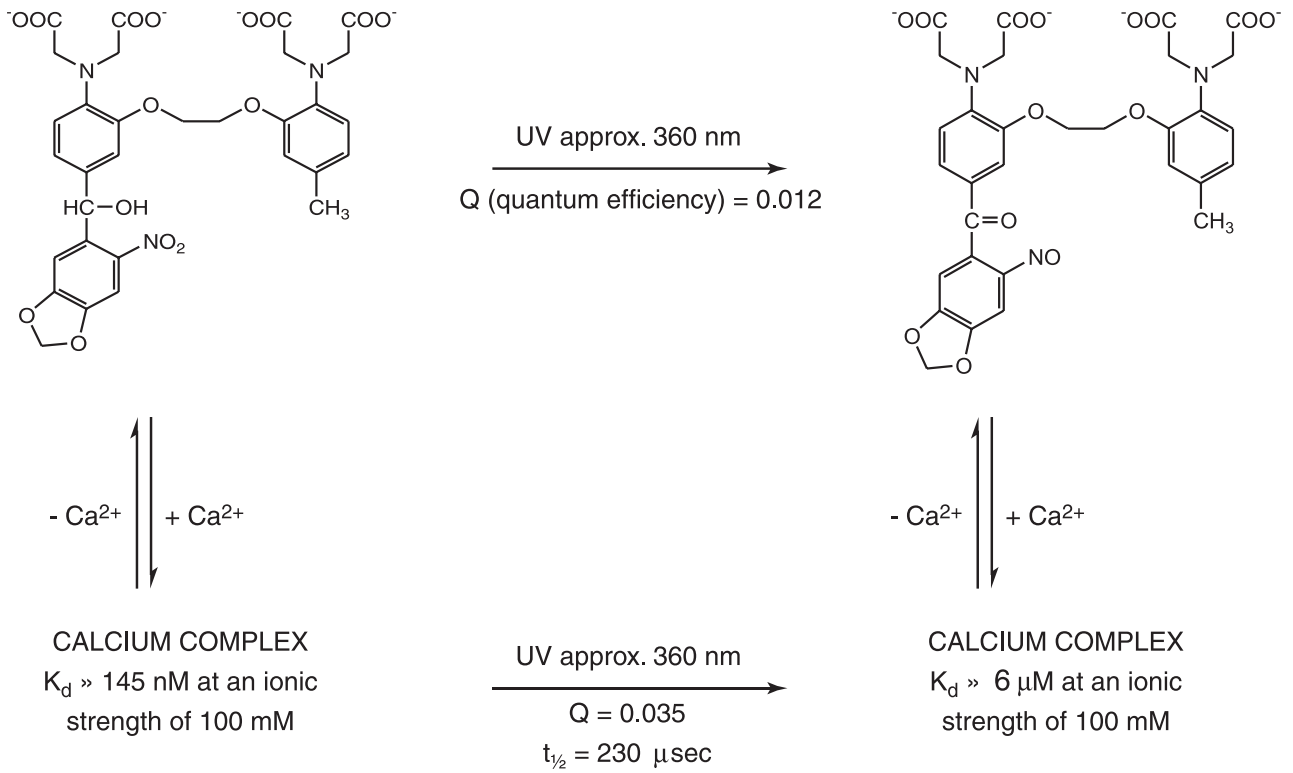
CAGED CALCIUM CHELATORS

Photolabile "caged" compounds are inert precursors of biologically active molecules that can be loaded into cells and subsequently released in their active form.[1] This is accomplished by a flash of light (~360 nm) that triggers the "uncaging" of the bioactive molecule. The absorption of photons excites the molecule to a higher (excited) energy state. The extra energy absorbed by the parent compound results in a dark reaction that leads to the formation of a stable physiologically active molecule. The duration and intensity of light can be varied with relative ease. The rate of photorelease varies widely among caged compounds; however, most reactions are complete within a few milliseconds. These molecules are highly advantageous in studying the intracellular kinetics of important signaling events such as activation of receptors and ion channels and release of neurotransmitters.[2,3]

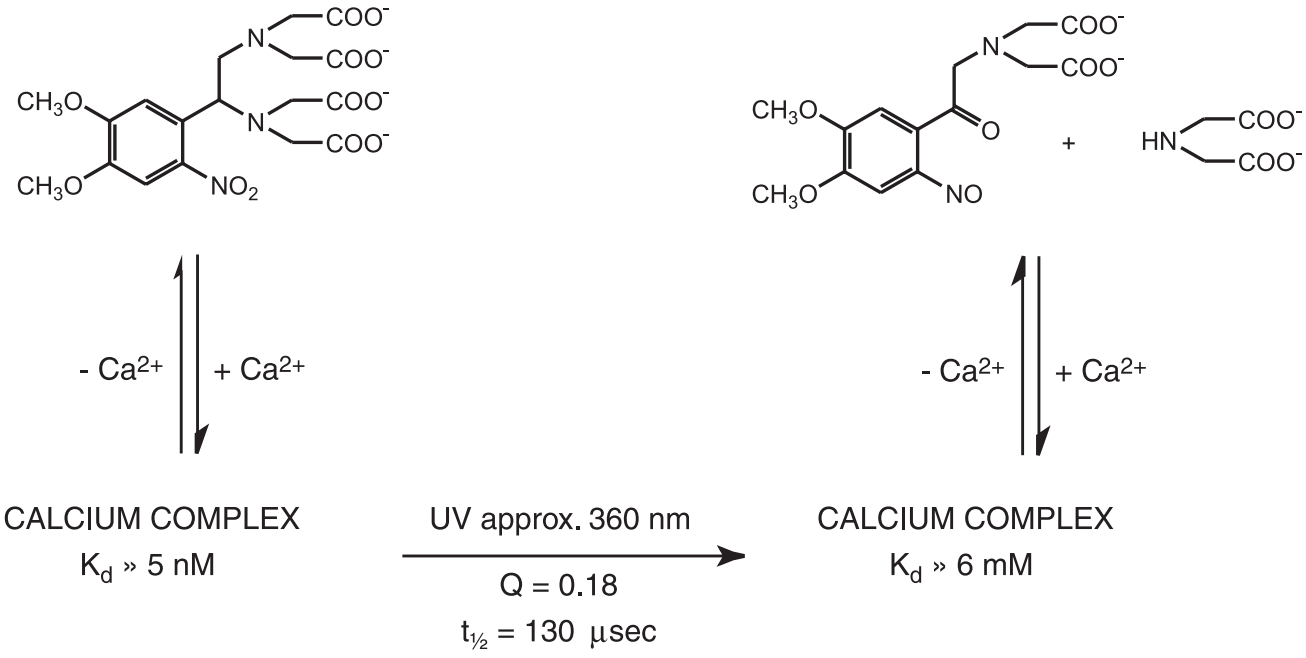
Most caged calcium chelators used to control the concentration of Ca^{2+} in the cell are based on the photochemistry of the *o*-nitrobenzyl group.[4] The photolysis of this group alters the affinity of these agents for Ca^{2+}. NITR 7, and DM-NITROPHEN™ Reagent are photolabile calcium chelators whose binding affinity for calcium is decreased by irradiation with light (~360 nm).[1] NITR compounds are designed structurally similar to BAPTA and become more electron-withdrawing after photolysis, resulting in reduced affinity for Ca^{2+} (Figure 4).[1,5] DM-NITROPHEN™ Reagent, on the other hand, is based around the chelator EDTA, which binds Ca^{2+} more tightly, and photolysis leads to a breakdown of EDTA to iminodiacetic acid fragments (Figure 5).[5] DM-NITROPHEN™ Reagent also exhibits significant Mg^{2+} binding, which may be a disadvantage when only intracellular Ca^{2+} is being studied.

References:

1. Adams, S.R., and Tsien, R.Y. 1993. *Annu. Rev. Physiol.* **55,** 755.
2. Somlyo, A.P., and Somylo, A.V. 1990. *Annu. Rev. Physiol.* **52,** 857.
3. Ogden, D.C., et al. 1990. *J. Physiol.* **422,** 585.
4. Gurney, A.M. 1991. in *Cellular Neurobiology: A Practical Approach* (Chad, J., and Wheal, H., eds) p. 153, IRL Press, Oxford.
5. Kaplan, J.H. 1990. *Annu. Rev. Physiol.* **52,** 897.



Characteristics of NITR 5



Chacteristics of DM-NITROPHEN™ Reagent

Ion Specificity. Ca^{2+} >> Mg^{2+}, H^+

Solubilities. The sodium salts are soluble in water (>10 mg/ml) and insoluble in acetone, alcohol, and ether. The free acids precipitate from aqueous solutions at pH < 5.0. The acetoxymethyl ester (NITR 5/AM) is soluble in DMSO but is insoluble in H_2O.

Storage. For prolonged storage, these compounds should be kept in a freezer under a dry, inert atmosphere and protected from light. Stock solutions should also be stored in a freezer under an inert atmosphere and protected from light.

Stability. The sodium salts are hygroscopic and can be exposed to subdued light for brief periods (weighing, transferring, etc.) without significant damage. Prolonged exposure to light will result in darkening of the solid and photolytic decomposition. Dispersions or dilute solutions of the acetoxymethyl ester (NITR 5/AM) in water or neutral buffers are stable for brief periods, but hydrolysis is accelerated under acid or alkaline conditions. Dilute solutions of these compounds must always be protected from light.

Comparison of the Spectral and Photochemical Properties of Calcium Chelators

Chelator	Dissociation Constant	Absorption Max. (Extinction Coefficient) ($M^{-1}cm^{-1}$)	Photolysis Quantum Efficiency (Q)	Time Constant of Calcium Release
NITR 5	145 nM	369 nm (4500)	0.035	0.23 msec
NITR 5, photolyzed	6 µM	390 nm (7000)	—	—
NITR 7	54 nM	369 nm (4500)	0.035	1.8 msec
NITR 7, photolyzed	3 µM	390 nm (7000)	—	—
DM-NITROPHEN™ Reagent	5 nM	345 nm (4200)	0.18	0.13 msec
DM-NITROPHEN™ Reagent, photolyzed	6 mM	295 nm (8200)	—	—