



Uncaging

Background

Caged compounds are biologically inactive substances with a photoactivatable group. Upon absorbing an appropriate photon the caged group can be cleaved and the active biological substance is released. With caged compounds it is for example possible to investigate second messengers independent of preceding signal transduction chain elements.

Several caging groups for a variety of biological substances are available. These caging groups vary in their spectral properties.

Caged substances range from ions, second messengers and amino acids to fluorescent dyes. Kits for caging carboxylic acids are also available.

Equipment

A light source with output in the UV-light range is needed. This can be a laser, mercury-lamp, UV-flash or even a monochromator. It has to be chosen whether only a part of the field of view needs to be illuminated, to control the release of the caged compound spatially, or whether the whole field of view needs to be illuminated for rapid and quantitative release of the caged compound.

TILL Photonics offers a UV-flash and the Polychrome V as light sources for uncaging.

One example for a method investigating second messengers and using caged compounds is:

Bedner P, Niessen H, Odermatt B, Willecke K, Harz H.

A method to determine the relative cAMP permeability of connexin channels.

2003 Exp Cell Res. 15;291(1):25-35.

Polychrome: A powerful light source for uncaging

High time resolution measurement of $[Ca^{2+}]_i$ and simultaneous release of calcium from DM-Nitrophen.

"Because the fluorescence excitation wavelength (of fura-2) also covers the UV range that can photolyze DMN (DM-Nitrophen), we can use the fluorescence excitation light to measure $[Ca^{2+}]_i$ and photorelease Ca^{2+} simultaneously. The R p (rate of Ca^{2+} release) for 380nm and 390nm excitation in our setup was found ... to be $1.497 \pm 0.005 s^{-1}$ and $0.966 \pm 0.050 s^{-1}$ (mean \pm S.D.), respectively." (See Reference 2 below, Xu et al.)



Comment: From the rate constants above you can calculate that 50% of the DM-Nitrophen will be photolyzed in 460ms (380nm) and 715ms(390nm). Higher rates of photolysis of DM-Nitrophen are reached at shorter wavelength (e.g. 350nm). Prof. Robert S. Zucker (Univ. of California at Berkeley) reported that the Polychrome II (a predecessor of the Polychrome V) at his setup could photolyse 50% of DM-Nitrophen in about **200ms**.

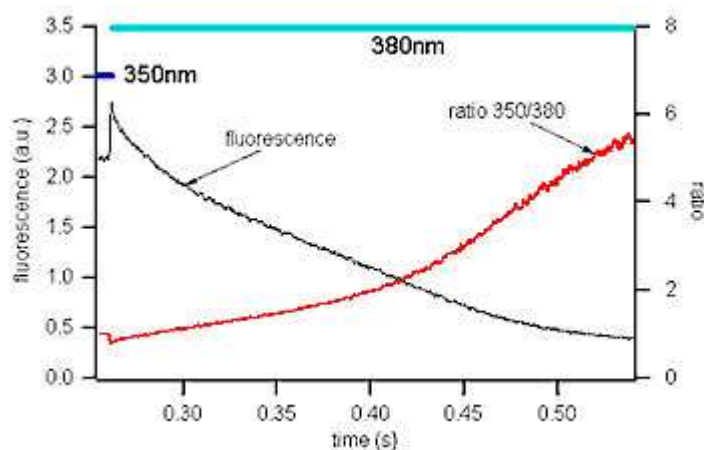


Figure 1: An example experiment using a TILL Polychrome for photolysis of DM-Nitrophen. fura-2 was the Ca^{2+} indicator. At the Ca^{2+} sensitive wavelength (here 380nm) of fura-2 the intensity of the fura excitation light is still so bright that Ca^{2+} was substantially released from DM-Nitrophen. This way you can elevate Ca^{2+} and measure its concentration simultaneously.

Read more about photolysis with steady light sources:

1. Zucker R.S. 1993. The calcium concentration clamp: spikes and reversible pulses using the photolabile chelator DM-nitrophen. *Cell Calcium* 14 :87-100
2. Xu T., Naraghi M., Kang H., Neher E. 1997. Kinetic studies of Ca^{2+} binding and Ca^{2+} clearance in the cytosol of adrenal chromaffin cells. *Biophys. J.* 73 :532-545

Uncaging experiments in combination with Photometry

Combined Electrophysiology and Flash/Photometry experiments were carried out at a setup running HEKA Pulse/PulseFit Software package and TILL UV-Flash and Photometry equipment. Fluorescence signals were usually acquired with the Fura Extension of Pulse and displayed in X-Chart (see Fig. 2). For high time resolution measurements fluorescence signals were sometimes sampled as 2nd sweep in Pulse (see inset of Figure 2).

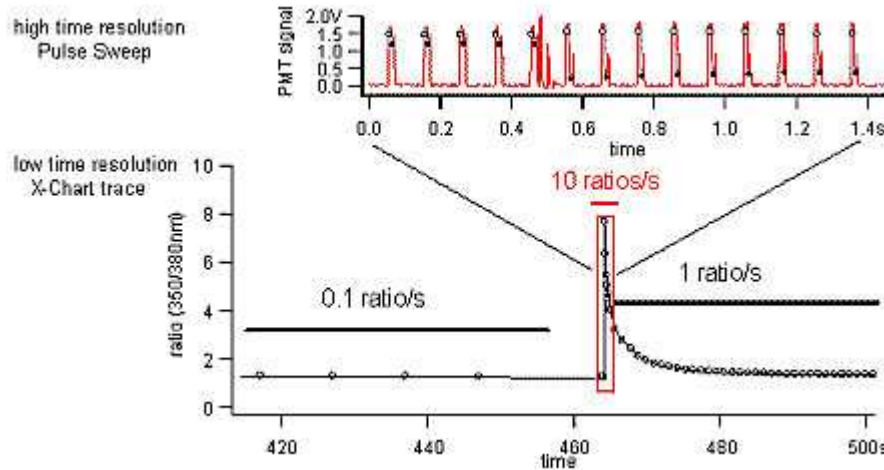


Figure 2: Flash/Photometry experiment running under HEKA Pulse Software.

Uncaging of Ca^{2+} from DM-Nitrophen

The TILL UV-Flash was coupled via a TILL 2-port epifluorescence condenser to a Zeiss Axiovert 100 microscope. The preparation was viewed through a Zeiss FLUAR 100x, 1.3, oil immersion objective.

A cell was loaded via the patch pipette with a solution containing: 4mM DM-Nitrophen, 2mM CaCl_2 , 0.2mM fura-2, 20mM Na-Hepes,...

A single full flash elevates intracellular free Ca^{2+} to a concentration that rapidly activates Ca^{2+} -dependent K^+ channels.

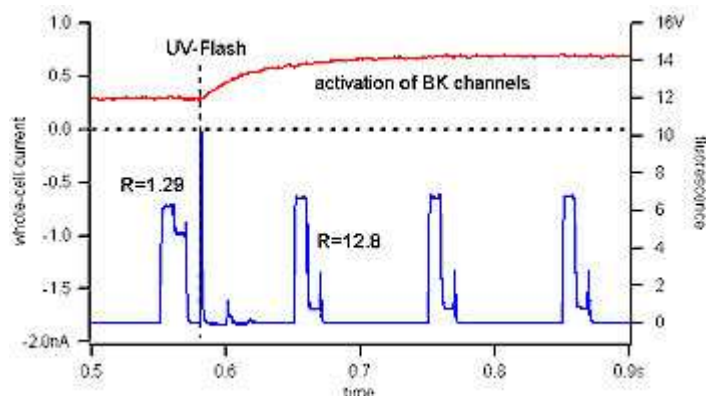


Figure 3: A single full flash elevates Ca^{2+} to several tenths of micromolar. The ratio changed from 1.29 to 12.8 (fura-2 is completely saturated), and BK-channels were rapidly activated.



Comment: The estimated free Ca^{2+} concentration was in the order of tenths of micromolars. Higher concentrations can easily be reached by increasing the Ca^{2+} -loading of DM-Nitrophen and its concentration.