Appendix B

The Berkeley Madonna model:

$\left[Ca^{2+}\right]_T, \left[Ca^{2+}\right]$, $\left[Ca^{2+}\right]_{t=0}$	total Ca^{2+} concentration (calc.), free Ca^{2+} concentration (sim.) and, initial free Ca^{2+} concentration (measured with Ca^{2+}-sensitive electrode),respectively
$\left[Mg^{2+}\right]_T, \left[Mg^{2+}\right]'$ $\left[Mg^{2+}\right]_{t=0}$	total Mg^{2+} concentration (known), free Mg^{2+} concentration (sim.) and, initial free Mg^{2+} concentration (calc.), respectively
$\left[DMn\right]_T, \left[DMn\right]'$ $\left[DMn\right]_{t=0}$	total DMn concentration (measured with photospectrometer), free DMn concentration (sim.) and, initial DMn concentration (calc.), respectively
$K_{d(CaDMn)}$	equilibrium dissociation constant of DMn with Ca^{2+} (fitted in set of initial experiments*)
$k_{on(CaDMn)}$	association rate constant of DMn with Ca^{2+} (calculated = $k_{off(CaDMn)} / K_{d(CaDMn)}$)
$k_{off(CaDMn)}$	dissociation rate constant of DMn with Ca^{2+} (fitted in set of initial experiments*)
$K_{d(MgDMn)}$	equilibrium dissociation constant of DMn with Mg^{2+} (fitted)
$k_{on(MgDMn)}$	association rate constant of DMn with Mg^{2+} (calculated = $k_{off(MgDMn)} / K_{d(MgDMn)}$)
$k_{off(MgDMn)}$	dissociation rate constant of DMn with Mg^{2+} (fitted)
$\left[CaDMn\right], \left[CaDMn\right]_{t=0}$	concentration of Ca^{2+}-DMn complex (sim.), and initial concentration (calc), resp.
$\left[MgDMn\right], \left[MgDMn\right]_{t=0}$	concentration of Mg^{2+}-DMn complex (sim.), and initial concentration (calc), resp.
$\left[PP1\right], \left[PP2\right]$	photoproduct concentration either formed by uncaging of CaDMn (PP1) or DMn or MgDMn (PP2), initial values are 0, (sim.)
$K_{d(CaPP1)}, K_{d(CaPP2)}$	equilibrium dissociation constant of PP1 and PP2, respectively, with Ca^{2+} (PP1 is fitted in set of initial experiments*, PP2 is fitted later)
$k_{off(CaPP1)}, k_{off(CaPP2)}$	equilibrium dissociation constant of PP1 and PP2, respectively, with Ca^{2+} (PP1 is fitted in set of initial experiments*, PP2 is fitted later)
$K_{d(MgPP)}$	equilibrium dissociation constant of both PP1 and PP2 with Mg^{2+} (fitted)
$k_{off(MgPP)}$	equilibrium dissociation constant of both PP1 and PP2 with Mg^{2+} (fitted)
$\left[CaPP1\right], \left[CaPP2\right]$	concentration of Ca^{2+}-PP1complex (sim.) and Ca^{2+}-PP2 complex (sim.), resp.
$\left[MgPP1\right], \left[MgPP2\right]$	concentration of Mg^{2+}-PP1 complex (sim.) and Mg^{2+}-PP2 complex (sim.), resp.
α, x	fraction of DM-n to be photolysed (fitted per trace), and its fraction that photolyzes with the fast time constant (fitted in set of initial experiments*).
$\left[DMn\right]_f, \left[CaDMn\right]_f$, $\left[MgDMn\right]_f$	total DMn concentration., total CaDMn concentration and, total MgDMn concentrations that photolyses with the fast timeconstant (all calc.), respectively
$\left[DMn\right]_s, \left[CaDMn\right]_s$, $\left[MgDMn\right]_s$	total DMn concentration., total CaDMn concentration and, total MgDMn concentrations that photolyses with the slow timeconstant (all calc.), respectively
τ_{fast}	time constant of fast photolysis (fitted in set of initial experiments*)
τ_{slow}	time constant of slow photolysis (fitted in set of initial experiments*)
$J_{fast(DMn)}, J_{fast(CaDMn)}$, $J_{fast(MgDMn)}$	flux/rate of fast uncaging to the various photoproducts and complexes of DMn, CaDMn and, MgDMn, respectively (all simulated)

$J_{slow(DMn)}, J_{slow(CaDMn)}, J_{slow(MgDMn)}$	flux/rate of slow uncaging to the various photoproducts and complexes of DMn, CaDMn and, MgDMn, respectively (all simulated)
t_{flash}	moment of initiation of photolysis (in these simulations 0)
$[D]_T$	total dye (Ca^{2+}-indicator) concentration = 100 μM OGB-5N
$[D]$	concentration of free dye (sim.)
$[CaD]$	concentration of Ca^{2+}-dye complex (sim.)
$K_{d(D)}$	equilibrium dissociation constant of the dye, OGB-5N = 29.3 μM (measured with Ca^{2+} solutions of known $[Ca^{2+}]$)
$k_{on(D)}$	association rate constant of the dye, OGB-5N with Ca^{2+} = $2.6 \cdot 10^8$ $M^{-1}s^{-1}$ (calculated = $k_{off(D)} / K_{d(D)}$)
$k_{off(D)}$	dissociation rate constant of the dye, OGB-5N with Ca^{2+} = 7.5×10^3 s^{-1} or 8.7×10^3 s^{-1} depending on the lot used (measured, derived from relaxation time constant of Ca^{2+}-dye complex after Ca^{2+}-pulse)
F_{ratio}	ratio F_{max} / F_{min} for the dye, OGB-5N = 10.8 or 40.0 depending on the lot used (measured with Ca^{2+} solutions of known $[Ca^{2+}]$)

DMn can be replaced everywhere for NP-EGTA to represent the uncaging model for NP-EGTA

The scheme of uncaging Ca^{2+} and Mg^{2+} and detecting Ca^{2+}

Pre flash

The kinetic reactions for the different fractions before photolysis are:

$$[DMn]+[Ca^{2+}] \underset{k_{off(CaDMn)}}{\overset{k_{on(CaDMn)}}{\rightleftarrows}} [CaDMn] \quad (1)$$

$$[DMn]+[Mg^{2+}] \underset{k_{off(MgDMn)}}{\overset{k_{on(MgDMn)}}{\rightleftarrows}} [MgDMn] \quad (2)$$

Flash photolysis of DM-nitrophen

Of the total DM-nitrophen (DMn) concentration (*$[DMn]_T$ = [DMn], [CaDMn], [MgDMn]*) a certain fraction α photolyzes. The flash energy of the UV laser determines the total proportion (α). Of this fraction α a fraction (*x*) photolyzes rapidly (*$[DM]_f$, $[CaDMn]_f$, $[MgDMn]_f$*) while a fraction (*1-x*) photolyzes slowly (*$[DMn]_s$, $[CaDMn]_s$, $[MgDMn]_s$*)

$$\left[DMn\right]_f = \alpha x\left[DMn\right] \tag{3}$$

$$\left[CaDMn\right]_f = \alpha x\left[CaDMn\right] \tag{4}$$

$$\left[MgDMn\right]_f = \alpha x\left[MgDMn\right] \tag{5}$$

$$\left[DMn\right]_s = \alpha\left(1-x\right)\left[DMn\right] \tag{6}$$

$$\left[CaDMn\right]_s = \alpha\left(1-x\right)\left[CaDMn\right] \tag{7}$$

$$\left[MgDMn\right]_s = \alpha\left(1-x\right)\left[MgDMn\right] \tag{8}$$

Where *x=1* for one uncaging timeconstant and *x≤1* for two uncaging timeconstants.

After the flash the affected DMn molecules transition into photoproducts of DMn (*[PP2]*), CaDMn (*[CaPP1]* and *[PP1]*) and, MgDMn (*[MgPP2]* and *[PP2]*) with time constants τ_{fast} and τ_{slow}:

$$\left[DMn\right]_f \xrightarrow{k=\frac{1}{\tau_{fast}}} 2\left[PP2\right] \xleftarrow{k=\frac{1}{\tau_{slow}}} \left[DMn\right]_s \tag{9}$$

$$\left[CaDMn\right]_f \xrightarrow{k=\frac{1}{\tau_{fast}}} \left([CaPP1]+\left[PP1\right]\right) \xleftarrow{k=\frac{1}{\tau_{slow}}} \left[CaDMn\right]_s \tag{10}$$

$$\left[MgDMn\right]_f \xrightarrow{k=\frac{1}{\tau_{fast}}} \left([MgPP2]+\left[PP2\right]\right) \xleftarrow{k=\frac{1}{\tau_{slow}}} \left[MgDMn\right]_s \tag{11}$$

In eq. 18 as Ca2+-free DMn is broken down into PP2 as described earlier by {Ayer, 1999 192 /id;Kaplan, 1988 179 /id}. However, based on our experiments it also be broken down into PP1.

Compared to DMn, the individual photoproducts may have different dissociation rate constants for Ca^{2+} and Mg^{2+} (leading to alternate affinities):

$$[PP1]+[Ca^{2+}]\underset{k_{off(CaPP1)}}{\overset{k_{on(CaDMn)}}{\rightleftarrows}}[CaPP1] \tag{12}$$

$$[PP2]+[Ca^{2+}]\underset{k_{off(CaPP2)}}{\overset{k_{on(CaDMn)}}{\rightleftarrows}}[CaPP2] \tag{13}$$

$$[PP1]+[Mg^{2+}]\underset{k_{off(MgPP)}}{\overset{k_{on(MgDMn)}}{\rightleftarrows}}[MgPP1] \tag{14}$$

$$[PP2]+[Mg^{2+}]\underset{k_{off(MgPP)}}{\overset{k_{on(MgDMn)}}{\rightleftarrows}}[MgPP2] \tag{15}$$

where we can note that the dissociation rates for PP1 and PP2 to Mg^{2+} are similar ($k_{off}MgPP$) whereas for Ca^{2+} they depend on the model used:

$$\textit{model (a):}\ k_{off}CaPP1 = k_{off}CaPP2 \tag{16}$$

$$\textit{model (b):}\ k_{off}CaPP1 \neq k_{off}CaPP2 \tag{17}$$

Determining [Ca^{2+}] with a Ca^{2+}-indicator

The kinetic reaction for the fluorescent dye (D) is:

$$[D]+[Ca^{2+}]\underset{k_{off(D)}}{\overset{k_{on(D)}}{\rightleftarrows}}[CaD] \tag{18}$$

In the prescence of both DMn and the dye there is a complete model for uncaging Ca^{2+} and Mg^{2+} and detecting Ca^{2+}.

Equations for uncaging Ca^{2+} and Mg^{2+} and detecting Ca^{2+}

For DMn

$$\frac{d[CaDMn]}{dt} = k_{on(CaDMn)} \cdot [Ca^{2+}] \cdot [DMn] - k_{off(CaDMn)} \cdot [CaDMn] - J_{fast(CaDMn)} - J_{slow(CaDMn)} \qquad (19)$$

$$\frac{d[MgDMn]}{dt} = k_{on(MgDMn)} \cdot [Mg^{2+}] \cdot [DMn] - k_{off(MgDMn)} \cdot [MgDMn] - J_{fast(MgDMn)} - J_{slow(MgDMn)} \qquad (20)$$

$$\begin{aligned}\frac{d[DMn]}{dt} = &-k_{on(CaDMn)} \cdot [Ca^{2+}] \cdot [DMn] + k_{off(CaDMn)} \cdot [CaDMn] \\ &- k_{on(MgDMn)} \cdot [Mg^{2+}] \cdot [DMn] + k_{off(MgDMn)} \cdot [MgDMn] - J_{fast(DMn)} - J_{slow(DMn)}\end{aligned} \qquad (21)$$

and

$$J_{fast(DMn)} = \frac{d[DMn]_f}{dt} = \frac{[DMn]_f}{\tau_{fast}} \cdot T_{flash} \qquad (22)$$

$$J_{slow(DMn)} = \frac{d[DMn]_s}{dt} = \frac{[DMn]_s}{\tau_{slow}} \cdot T_{flash} \qquad (23)$$

$$J_{fast(CaDMn)} = \frac{d[CaDMn]_f}{dt} = \frac{[CaDMn]_f}{\tau_{fast}} \cdot T_{flash} \qquad (24)$$

$$J_{slow(CaDMn)} = \frac{d[CaDMn]_s}{dt} = \frac{[CaDMn]_s}{\tau_{slow}} \cdot T_{flash} \qquad (25)$$

$$J_{fast(MgDMn)} = \frac{d[MgDMn]_f}{dt} = \frac{[MgDMn]_f}{\tau_{fast}} \cdot T_{flash} \qquad (26)$$

$$J_{slow(MgDMn)} = \frac{d[MgDMn]_s}{dt} = \frac{[MgDMn]_s}{\tau_{slow}} \cdot T_{flash} \qquad (27)$$

where $T_{flash} = 0$ if $t < t_{flash}$ (28)

and $T_{flash} = 1$ if $t \geq t_{flash}$ (29)

For the photoproducts:

$$\frac{d[CaPP1]}{dt} = k_{on(CaDMn)} \cdot \left[Ca^{2+}\right] \cdot [PP1] - k_{off(CaPP1)} \cdot [CaPP1] + J_{fast(CaDMn)} + J_{slow(CaDMn)} \tag{30}$$

$$\frac{d[CaPP2]}{dt} = k_{on(CaDMn)} \cdot \left[Ca^{2+}\right] \cdot [PP2] - k_{off(CaPP2)} \cdot [CaPP2] \tag{31}$$

$$\frac{d[MgPP1]}{dt} = k_{on(MgDMn)} \cdot \left[Mg^{2+}\right] \cdot [PP1] - k_{off(MgPP)} \cdot [MgPP1] \tag{32}$$

$$\frac{d[MgPP2]}{dt} = k_{on(MgDMn)} \cdot \left[Mg^{2+}\right] \cdot [PP2] - k_{off(MgPP)} \cdot [CaPP2] + J_{fast(MgDMn)} + J_{slow(MgDMn)} \tag{33}$$

$$\begin{aligned} \frac{d[PP1]}{dt} = & -k_{on(CaDMn)} \cdot \left[Ca^{2+}\right] \cdot [PP1] + k_{off(CaPP1)} \cdot [CaPP1] \\ & - k_{on(MgDMn)} \cdot \left[Mg^{2+}\right] \cdot [PP1] + k_{off(MgPP)} \cdot [MgPP1] \\ & + J_{fast(CaDMn)} + J_{slow(CaDMn)} \end{aligned} \tag{34}$$

$$\begin{aligned} \frac{d[PP2]}{dt} = & -k_{on(CaDMn)} \cdot \left[Ca^{2+}\right] \cdot [PP2] + k_{off(CaPP2)} \cdot [CaPP2] \\ & - k_{on(MgDMn)} \cdot \left[Mg^{2+}\right] \cdot [PP2] + k_{off(MgPP)} \cdot [MgPP2] \\ & + 2 \cdot J_{fast(DMn)} + 2 \cdot J_{slow(DMn)} + \cdot J_{fast(MgDMn)} + \cdot J_{slow(MgDMn)} \end{aligned} \tag{35}$$

For the dye

$$\frac{d[CaD]}{dt} = k_{on(D)} \cdot \left[Ca^{2+}\right] \cdot [D] - k_{off(D)} \cdot [CaD] \tag{36}$$

$$[D] = [D]_T - [CaD] \tag{37}$$

For Ca^{2+} and Mg^{2+}

$$\left[Ca^{2+}\right] = \left[Ca^{2+}\right]_T - [CaDMn] - [CaPP1] - [CaPP2] \tag{38}$$

$$\left[Ca^{2+}\right]_T = \left[Ca^{2+}\right]_{t=0} + [CaDMn]_{t=0} \tag{39}$$

$$\left[Mg^{2+}\right] = \left[Mg^{2+}\right]_T - [MgDMn] - [MgPP1] - [MgPP2] \tag{40}$$

Initial conditions

$$\left[DMn\right]_{t=0} = \left[DMn\right]_T - \left[CaDMn\right]_{t=0} - \left[MgDMn\right]_{t=0} \tag{41}$$

$$\left[CaDMn\right]_{t=0} = \frac{K_{d(MgDMn)} \cdot \left[Ca^{2+}\right]_{t=0}}{K_{d(MgDMn)} \cdot \left[Ca^{2+}\right]_{t=0} + K_{d(CaDMn)} \cdot \left[Mg^{2+}\right]_{t=0} + K_{d(CaDMn)} \cdot K_{d(MgDMn)}} \cdot \left[DMn\right]_T \tag{42}$$

$$\left[MgDMn\right]_{t=0} = \frac{K_{d(CaDMn)} \cdot \left[Mg^{2+}\right]_{t=0}}{K_{d(CaDMn)} \cdot \left[Mg^{2+}\right]_{t=0} + K_{d(MgDMn)} \cdot \left[Ca^{2+}\right]_{t=0} + K_{d(CaDMn)} \cdot K_{d(MgDMn)}} \cdot \left[DMn\right]_T \tag{43}$$

$$\left[Mg^{2+}\right] = \frac{K_{d(CaDMn)} \cdot \left[Mg^{2+}\right]_T - K_{d(MgDMn)}\left(K_{d(CaDMn)} - \left[Ca^{2+}\right] - \left[DMn\right]_T\right) + X}{2K_{d(CaDMn)}} \tag{44a}$$

$$X = \sqrt{\begin{aligned} &K_{d(CaDMn)}^{\;2}\left(\left[Mg^{2+}\right]_T\left(\left[Mg^{2+}\right]_T + 2\cdot K_{d(MgDMn)} - 2\cdot\left[DMn\right]_T\right) + \left[DMn\right]_T\left(\left[DMn\right]_T + 2\cdot K_{d(MgDMn)}\right) + K_{d(MgDMn)}^{\;2}\right) \\ &+ K_{d(MgDMn)} \cdot \left[Ca^{2+}\right]\left(K_{d(MgDMn)} \cdot \left[Ca^{2+}\right] + 2\cdot K_{d(CaDMn)}\left(\left[Mg^{2+}\right]_T + K_{d(MgDMn)}\left[DMn\right]_T\right)\right)\end{aligned}} \tag{44b}$$

(also see appendix A, see online journal)

Initially there are no photoproducts

$$\left[CaD\right]_{t=0} = \frac{\left[Ca^{2+}\right]_{t=0}}{K_{d(D)} + \left[Ca^{2+}\right]_{t=0}} \cdot \left[D\right]_T \tag{45}$$

Model output

$$\frac{\Delta F}{F} = \frac{\Delta F(t)}{F_{t=0}} = \frac{\left[CaD\right] \cdot \left(F_{ratio} - 1\right) + \left[D\right]_T}{\left[CaD\right]_{t=0} \cdot \left(F_{ratio} - 1\right) + \left[D\right]_T} \tag{46}$$