

Appendix B

The Berkeley Madonna model:

$[Ca^{2+}]_T, [Ca^{2+}]_{t=0}$	total Ca^{2+} concentration (calc.), free Ca^{2+} concentration (sim.) and, initial free Ca^{2+} concentration (measured with Ca^{2+} -sensitive electrode), respectively
$[Mg^{2+}]_T, [Mg^{2+}]_{t=0}$	total Mg^{2+} concentration (known), free Mg^{2+} concentration (sim.) and, initial free Mg^{2+} concentration (calc.), respectively
$[DMn]_T, [DMn]_{t=0}$	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)
$[CaDMn]_{t=0}, [CaDMn]_{t=0}$	concentration of Ca^{2+} -DMn complex (sim.), and initial concentration (calc), resp.
$[MgDMn]_{t=0}, [MgDMn]_{t=0}$	concentration of Mg^{2+} -DMn complex (sim.), and initial concentration (calc), resp.
$[PP1], [PP2]$	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)
$[CaPP1], [CaPP2]$	concentration of Ca^{2+} -PP1 complex (sim.) and Ca^{2+} -PP2 complex (sim.), resp.
$[MgPP1], [MgPP2]$	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*).
$[DMn]_f, [CaDMn]_f, [MgDMn]_f$	total DMn concentration., total CaDMn concentration and, total MgDMn concentrations that photolyses with the fast timeconstant (all calc.), respectively
$[DMn]_s, [CaDMn]_s, [MgDMn]_s$	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)

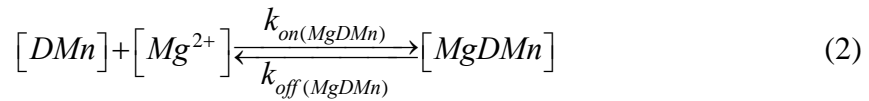
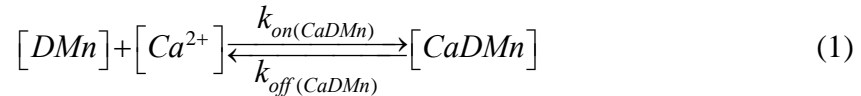
$J_{slow(DMn)}, J_{slow(CaDMn)}, J_{slow(MgDMn)}$	flux/rate of slow uncaging to the various photoproducts and complexes of DMn, CaDMn and, MgDMn, respectively (all simulated)
t_{flash}	moment of initiation of photolysis (in these simulations 0)
$[D]_T$	total dye (Ca^{2+} -indicator) concentration = 100 μ M 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 μ M (measured with Ca^{2+} solutions of known $[Ca^{2+}]$)
$k_{on(D)}$	association rate constant of the dye, OGB-5N with $Ca^{2+} = 2.6 \cdot 10^8 \text{ M}^{-1}\text{s}^{-1}$ (calculated = $k_{off(D)} / K_{d(D)}$)
$k_{off(D)}$	dissociation rate constant of the dye, OGB-5N with $Ca^{2+} = 7.5 \times 10^3 \text{ s}^{-1}$ or $8.7 \times 10^3 \text{ s}^{-1}$ 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:



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 ($[DMn]_s, [CaDMn]_s, [MgDMn]_s$)

$$[DMn]_f = \alpha x [DMn] \quad (3)$$

$$[CaDMn]_f = \alpha x [CaDMn] \quad (4)$$

$$[MgDMn]_f = \alpha x [MgDMn] \quad (5)$$

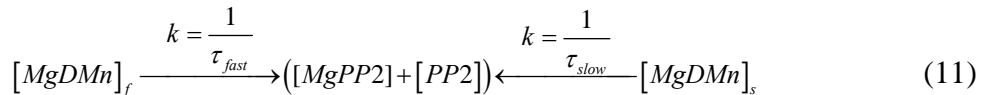
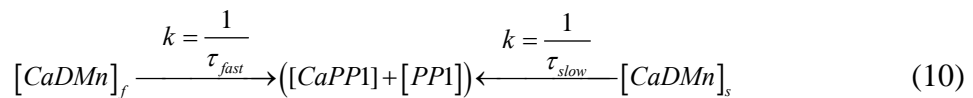
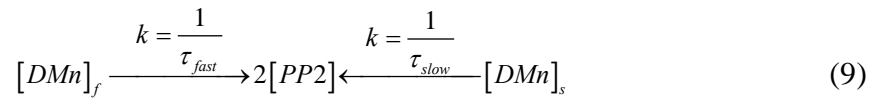
$$[DMn]_s = \alpha (1-x) [DMn] \quad (6)$$

$$[CaDMn]_s = \alpha (1-x) [CaDMn] \quad (7)$$

$$[MgDMn]_s = \alpha (1-x) [MgDMn] \quad (8)$$

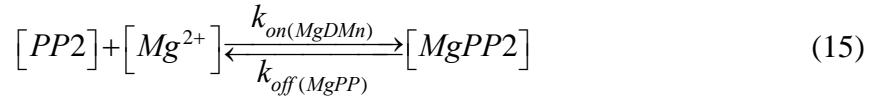
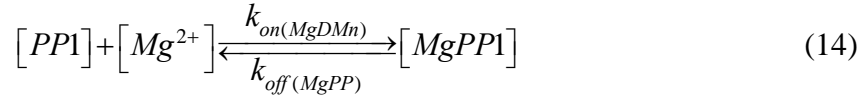
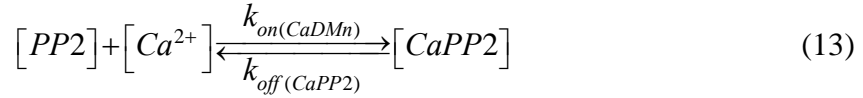
Where $x=1$ for one uncaging timeconstant and $x \leq 1$ for two uncaging timeconstants.

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} :



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 Ca²⁺ and Mg²⁺ (leading to alternate affinities):



where we can note that the dissociation rates for PP1 and PP2 to Mg^{2+} are similar ($k_{offMgPP}$)

whereas for Ca^{2+} they depend on the model used:

$$\text{model (a): } k_{offCaPP1} = k_{offCaPP2} \quad (16)$$

$$\text{model (b): } k_{offCaPP1} \neq k_{offCaPP2} \quad (17)$$

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

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



In the presence 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

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

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

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

and

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

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

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

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

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

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

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

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

For the photoproducts:

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

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

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

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

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

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

For the dye

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

$$[D] = [D]_T - [CaD] \quad (37)$$

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

$$[Ca^{2+}] = [Ca^{2+}]_T - [CaDMn] - [CaPP1] - [CaPP2] \quad (38)$$

$$[Ca^{2+}]_T = [Ca^{2+}]_{t=0} + [CaDMn]_{t=0} \quad (39)$$

$$[Mg^{2+}] = [Mg^{2+}]_T - [MgDMn] - [MgPP1] - [MgPP2] \quad (40)$$

Initial conditions

$$[DMn]_{t=0} = [DMn]_T - [CaDMn]_{t=0} - [MgDMn]_{t=0} \quad (41)$$

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

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

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

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

(also see appendix A, see online journal)

Initially there are no photoproducts

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

Model output

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