Supporting information

Insights on the interaction of calcein with calcium carbonate and implications in biomineralization studies

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Experimental setup

Crystal growth experiments

Calcite precipitation Calcium chloride dehydrate was purchased from Fluka, magnesium chloride hexahydrate was purchased from Sigma Aldrich and anhydrous ammonium carbonate was purchased from Acros Organics. All reagents were ACS grade and used as purchased. Calcium carbonate crystals were prepared using the vapour diffusion technique, consisting in the diffusion of NH_3 and CO_2 vapour obtained from the decomposition of 3.5 g of $(NH_4)_2CO_3$ into 750 µL of 10 µM $CaCl_2$ solution contained in a 24-well multiwell dish containing glass coverslips and covered with aluminium foil with a hole of ø1 mm on each well in a closed desiccator. The appropriate amount of additive was added to each well. The precipitation process was allowed to proceed for 4 days, after which each well was washed 3 times with DI water and once with ethanol.

Aragonite precipitation Calcium chloride dehydrate was purchased from Fluka, magnesium chloride hexahydrate was purchased from Sigma Aldrich and anhydrous ammonium carbonate was purchased from Acros Organics. All reagents were ACS grade and used as purchased. Calcium carbonate crystals were prepared using the vapour diffusion technique, consisting in the diffusion of NH_3 and CO_2 vapour obtained from the decomposition of 3.5 g of $(NH_4)_2CO_3$ into 750 µL of 10 µM $CaCl_2$ solution contained in a 24-well multiwell dish containing glass coverslips and covered with aluminium foil with a hole of ø1 mm on each well in a closed desiccator. The appropriate amount of additive was added to each well. To obtain aragonite $MgCl_2$ was added to $CaCl_2$ to obtain a 4:1 Mg:Ca ratio. The precipitation process was allowed to proceed for 4 days, after which each well was washed 3 times with DI water and once with ethanol. Mg^{2+} favours the precipitation of aragonite by (i) adsorbing on calcite nuclei and preventing the integration of Ca^{2+} in the lattice and (ii) forming magnesium calcite, which is more soluble than calcite and as soluble as aragonite.

Confocal imaging A Leica TCS SL microscope was used to perform confocal microscopy, using the software "Leica confocal software". Samples for confocal imaging were precipitated directly onto glass coverslip as described above and mounted on glass slides by using Canada balsam (Sigma Aldrich-C1795). Samples were irradiated with 488 nm laser and emission was observed in the 520 nm-700 nm range. Slices were collected in z-stack mode and stacked to obtain the presented images.

Calcein loading 750 µL of 0.1 M citrate buffer pH 4.5 were added to each well in order to dissolve the crystals. Dissolution was allowed to proceed for 4 hours on a rocking plane. Absorbance spectra were collected using an Agilent Cary 300-Bio UV-Vis spectrophotometer and calcium content was measured with flame atomic absorption spectroscopy (Perkin-Elmer AAnalyst 100). The calcein content was computed by dividing the quantity of calcein obtained from the UV-Vis spectra by the calcium carbonate quantity computed by calcium measures with atomic absorption.

High resolution X-Ray diffraction High resolution X-Ray diffractogram were collected at Grenoble synchrotron using a wavelength of 0.4959 Å. Diffractogram were converted to Cu Kα wavelength (1.5406 Å) for easier understanding.

SEM imaging SEM images were collected using a HR-SEM (ULTRA Plus, Zeiss, Oberkochen, Germany) after coating the samples with 3 nm of gold.

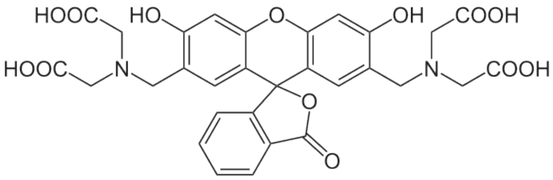

Figure SI1. Calcein structure.

Confocal imaging

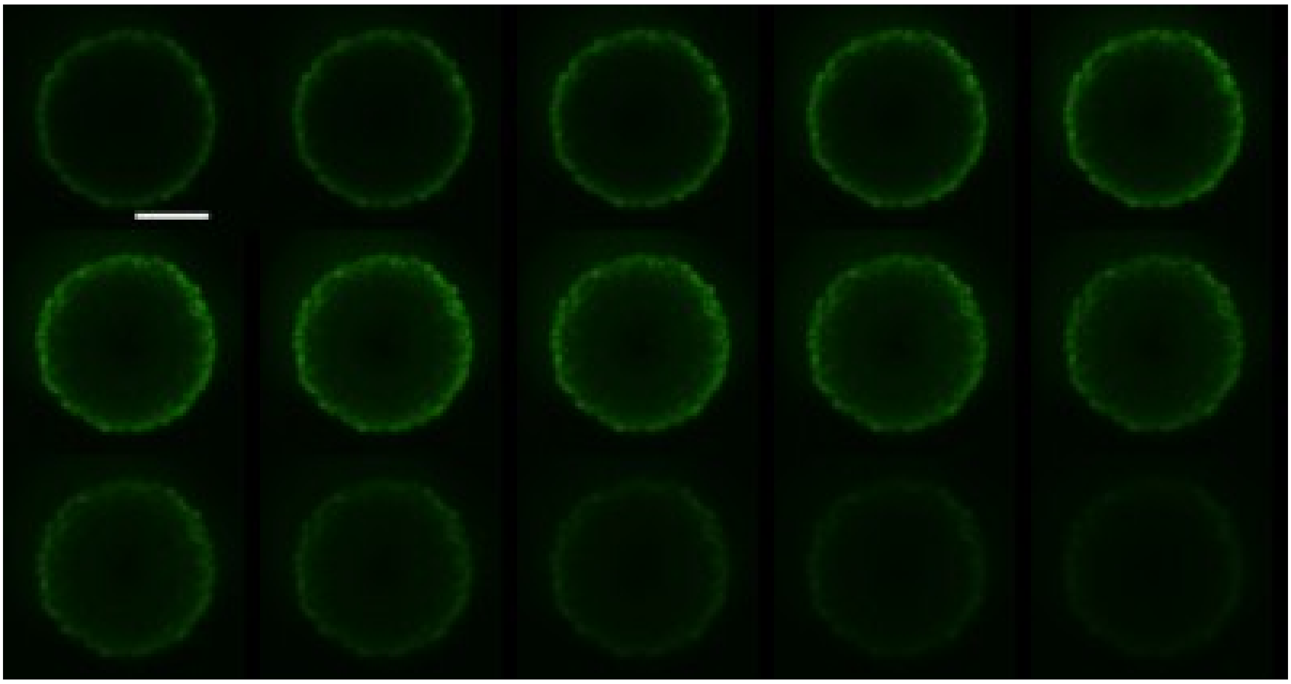

Figure SI2. Confocal imaging of a calcite crystal grown in the presence of 400 µM calcein. Scalebar is 30 µM.

Calcium content measurements

Table SI1. Ca^{2+} content measured with flame absorption atomic spectroscopy

Calcein (µM)	Aragonite				Calcite			
	pristine		bleached		pristine		bleached	
	loading (mg/L)	st. dev. (mg/L)	loading (mg/L)	st. dev. (mg/L)	loading (mg/L)	st. dev. (mg/L)	loading (mg/L)	st. dev. (mg/L)
blank	218.37	26.01	212.34	1.98	209.20	6.41	191.61	10.12
4	235.26	7.58	213.87	16.12	225.25	6.41	223.68	5.13
40	243.92	12.65	227.25	2.30	182.27	29.74	212.51	11.82
400	214.90	13.35	214.77	4.09	136.71	12.91	139.54	14.10

Calcein loading relative to the starting solution concentration

Table SI2. Calcein loading into aragonite and calcite crystals relative to calcein concentration in the crystallization solution.

Calcein (µM)	Aragonite		Calcite	
	loading (%)	st. dev. (%)	loading (%)	st. dev. (%)
4	9.67	1.91	5.81	0.41
40	6.89	0.34	7.30	0.95
400	4.44	0.79	3.27	0.20

Table SI3. Calcein loading (wt.%) into aragonite and calcite crystals relative to the mass of calcium carbonate precipitated.

Calcein (µM)	Aragonite				Calcite			
	pristine		bleached		pristine		bleached	
	loading (wt.%)	st. dev. (wt.%)	loading (wt.%)	st. dev. (wt.%)	loading (wt.%)	st. dev. (wt.%)	loading (wt.%)	st. dev. (wt.%)
blank*	0.02	0.01	0.04	0.02	0.01	0.00	0.03	0.00
4	0.04	0.01	0.02	0.00	0.03	0.00	0.03	0.00
40	0.26	0.02	0.15	0.01	0.33	0.04	0.30	0.02
400	2.33	0.31	1.71	0.12	2.60	0.11	2.54	0.04

* This sample does not contain calcein.

Low magnification SEM images

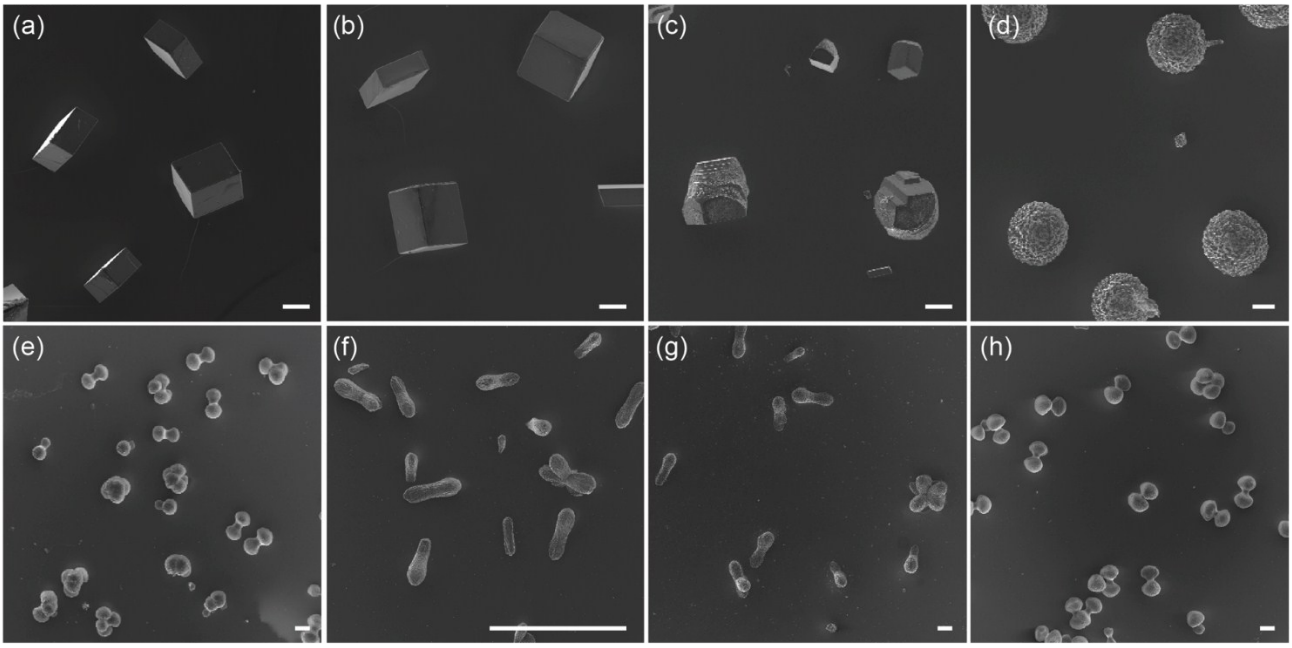

Figure SI3. SEM images of crystals grown with 10 mM Ca^{2+} (a) without additives and in the presence of (b) 4 µM. (c) 40 µM and (d) 400 µM calcein and crystals grown with 10 mM Ca^{2+} and 40 mM Mg^{2+} (e) without additives and in the presence of (f) 4 µM. (g) 40 µM and (h) 400 µM. Scale bar: 20 µm.

High resolution X-ray powder diffraction patterns

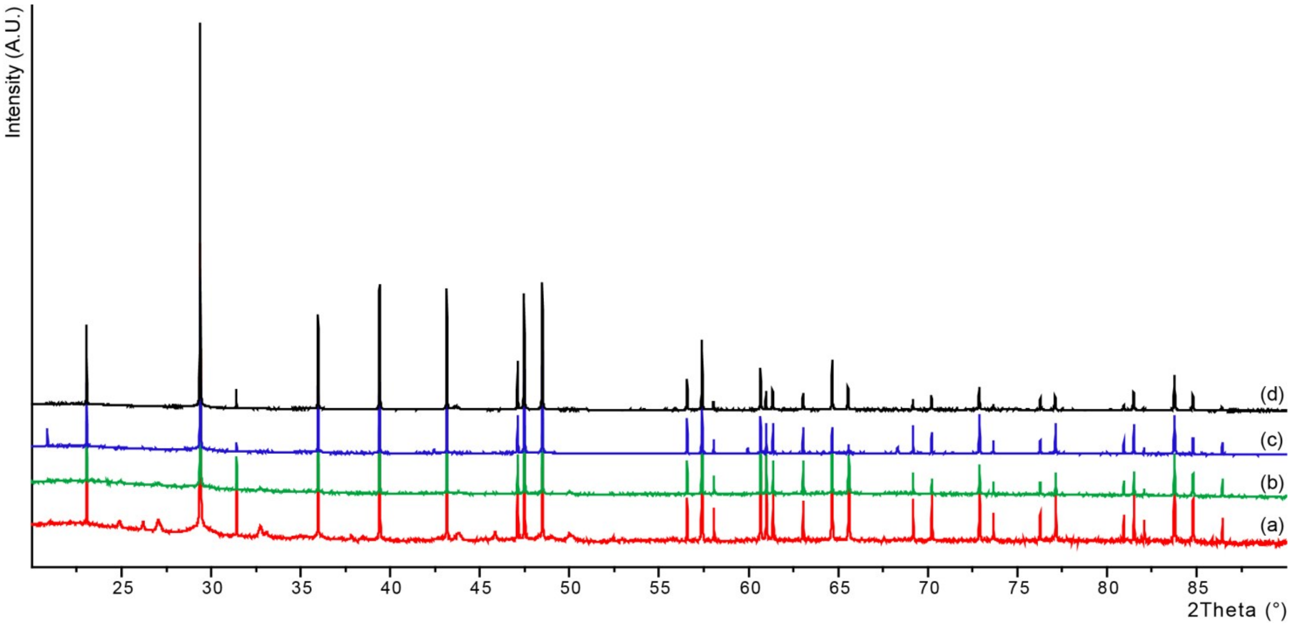

Figure SI4. High resolution X-ray powder diffraction patterns of calcite crystals grown (a) without additives and in the presence of (b) 4 µM (c) 40 µM and (d) 400 µM calcein. Wavelength converted from 0.4959 Å to 1.5406 Å.

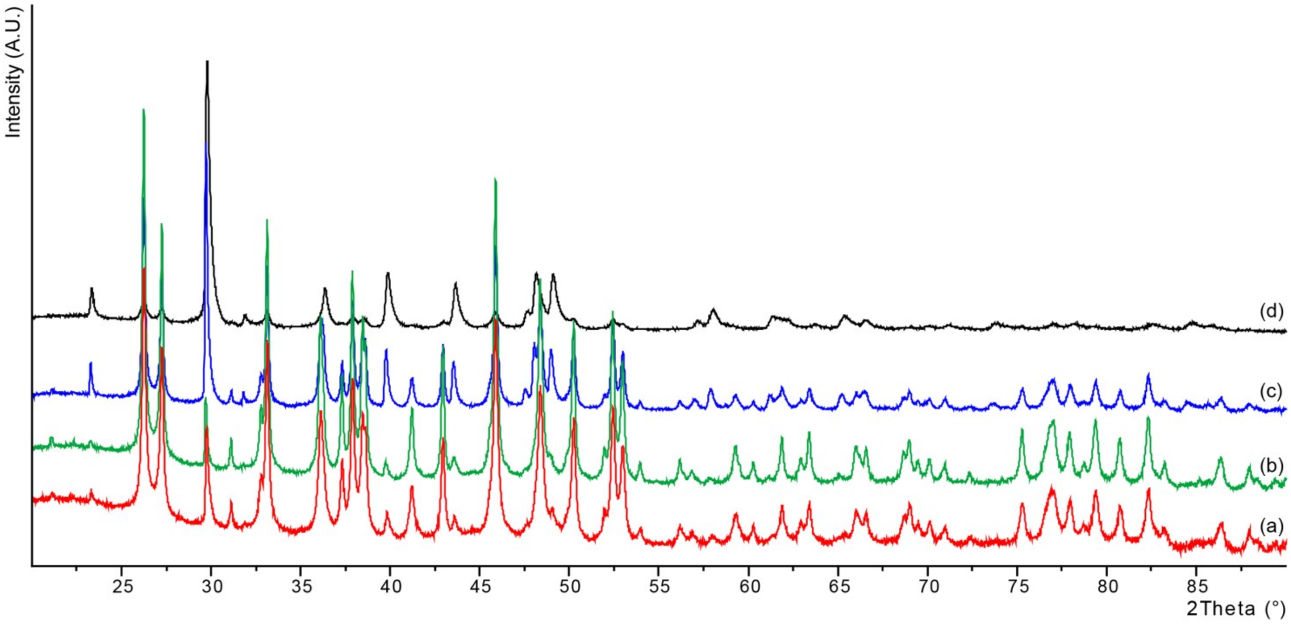

Figure SI5. High resolution X-ray powder diffraction patterns of crystals grown from a solution containing 40 mM Mg^{2+} and 10 mM Ca^{2+} (a) without additives and in the presence of (b) 4 µM (c) 40 µM and (d) 400 µM calcein. Wavelength converted from 0.4959 Å to 1.5406 Å.

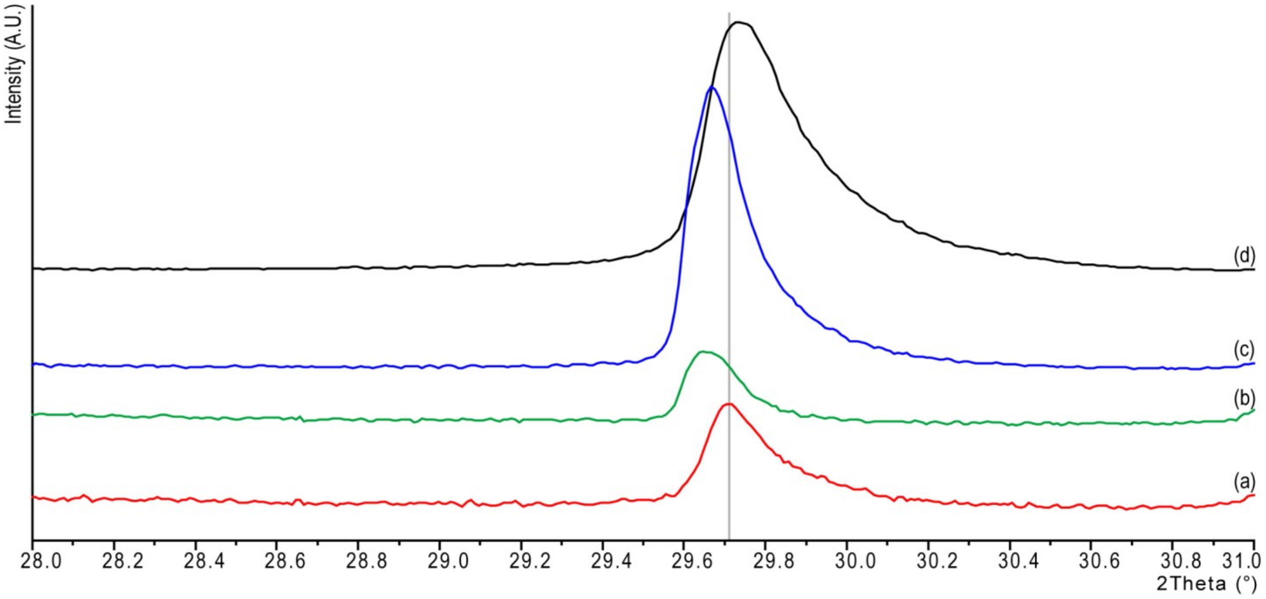

Figure SI6. High resolution X-ray powder diffraction patterns in the 28 – 31 2θ(°) range of magnesium calcite crystals grown from a solution containing 40 mM Mg^{2+} and 10 mM Ca^{2+} (a) without additives and in the presence of (b) 4 µM (c) 40 µM and (d) 400 µM calcein. Wavelength converted from 0.4959 Å to 1.5406 Å.

Rietveld analysis results

Table SI4. Aragonite and Mg-calcite content of samples grown in the presence of 40 mM Mg^{2+} and 10 mM Ca^{2+} in the presence of different calcein concentrations.

Calcein / µM	Aragonite / wt.%	Mg-calcite / wt.%
blank	93	7
4	98	2
40	76	24
400	4	96

Table SI5. Cell parameters and distorsions of calcite crystals grown in the presence of different calcein concentrations.

	Blank	4 µM	40 µM	400 µM
a, Å	4.99053(5)	4.99059(4)	4.99066(6)	4.99048(2)
distortions, a		*1.0E-05*	*2.4E-05*	*-1.2E-05*
c, Å	17.06772(8)	17.06868(8)	17.0713(1)	17.07949(4)
distortions, c		*5.6E-05*	*2.1E-04*	*6.9E-04*

Table SI6. Cell parameters and distorsions of aragonite and Mg-calcite crystals grown in the presence of different calcein concentrations.

	Blank		4 µM		40 µM		400 µM	
phase	Aragonite	Mg-calcite	Aragonite	Mg-calcite	Aragonite	Mg-calcite	Aragonite	Mg-calcite
a, Å	4.9626(1)	4.943(2)	4.96340(8)	4.9489(7)	4.9628(1)	4.9503(4)	4.9566(8)	4.937(5)
distortions, a			*1.6E-04*	*1.2E-03*	*4.0E-05*	*1.5E-03*	*-1.2E-03*	*-1.2E-03*
b, Å	7.9680(2)	4.943(2)	7.9687(1)	4.9489(7)	7.9683(2)	4.9503(4)	7.985(2)	4.937(5)
distortions, b			*8.8E-05*	*1.2E-03*	*-6.5E-04*	*1.5E-03*	*2.1E-03*	*-1.2E-03*
c, Å	5.7515(1)	16.851(3)	5.75352(8)	16.907(2)	5.7517(1)	16.8794(7)	5.7487(9)	17.14(13)
distortions, c			*3.5E-04*	*3.3E-03*	*3.5E-05*	*1.7E-03*	*-4.9E-04*	*1.7E-02*