

1 **Supplementary Materials**

2 **1. Experimental method**

3 **1.1. Synthesis of CaCO₃ *ex situ***

4 The syntheses of CaCO₃ were performed *ex situ* to estimate the reaction time to
5 implement the *in situ* TEM experiments. Additionally, a comparison between *in situ* and
6 *ex situ* TEM allowed us to understand the influence of the electron beam-induced
7 damage/artifacts in the liquid environment.

8 For *ex situ* TEM, the syntheses were performed on a TEM grid and observed by
9 conventional TEM. The experiments were conducted by dropping 5 μL of Na₂CO₃ with
10 and without L-Asp on a copper grid with carbon film followed by a drip of CaCl₂ 5 μL.
11 The reaction was stopped by washing 3 times with distilled water for times varying
12 from 30 seconds to 30 min. The grids were analyzed using a JEOL 2100F transmission
13 electron microscope equipped with a JED-2300 energy dispersive spectrometer (JEOL).
14 Images were treated using Gatan DigitalMicrograph and Analysis Station (JEOL)
15 software.

16 **1.2. X-ray diffraction characterization**

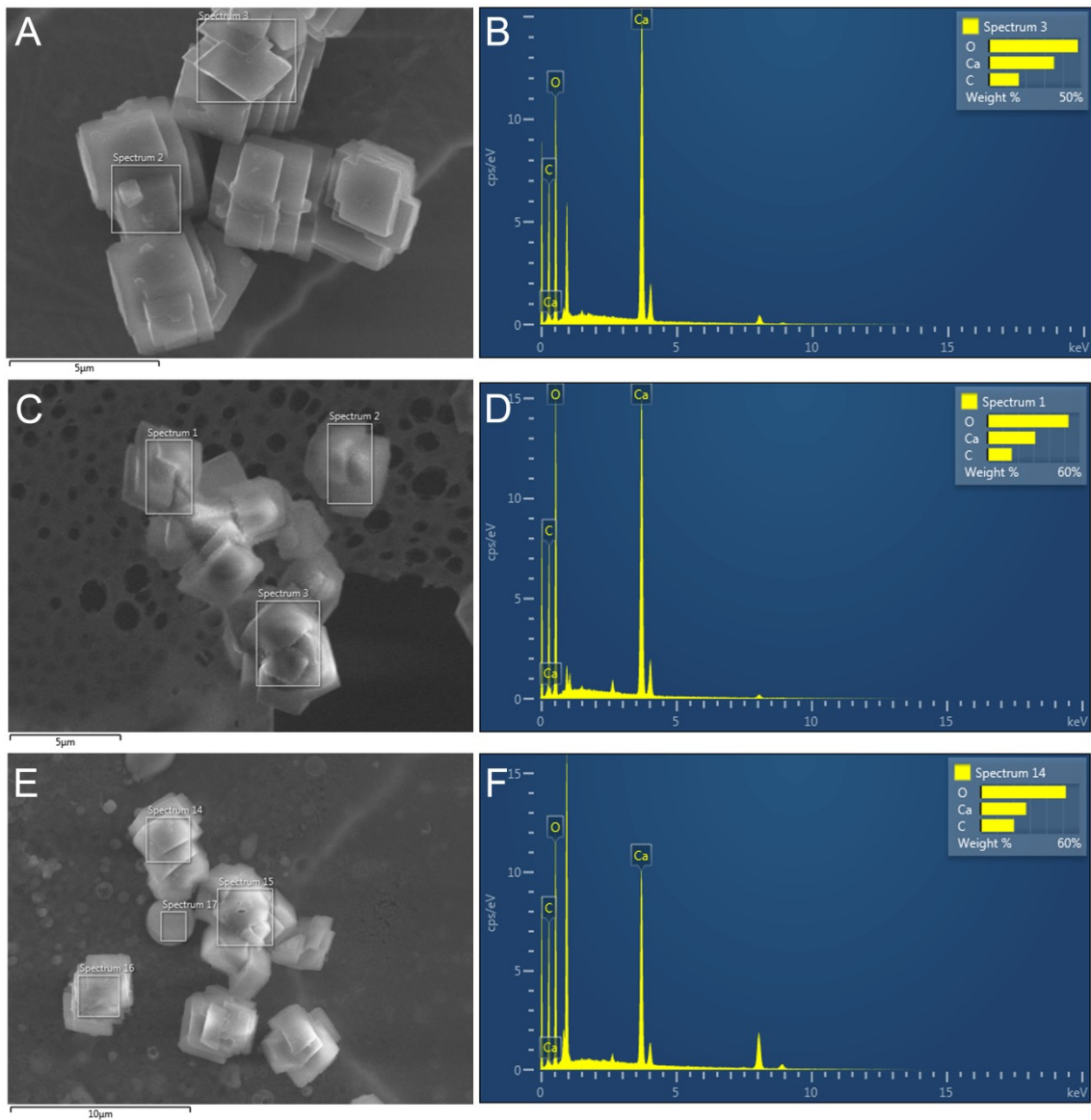
17 In order to confirm the polymorph formed during our experiments, 100 mM calcium
18 carbonate was added to a beaker containing 100 mM Na₂CO₃ (control experiment) and
19 100 mM Na₂CO₃ with 100 mM L-asp (L-Asp experiment). The reactions took place
20 under magnetic stirring, respecting the 1:1 stoichiometric ratio. Then, both samples
21 were centrifuged at 5000 rpm for 5 min 3 times and washed with distilled water
22 between each cycle, and lyophilized for analysis by x-ray diffraction. The X-ray
23 diffractogram of the dried samples were obtained using an X'Pert PRO diffractometer

1 (PANanalytical) equipped with x'celerator detector (PANanalytical), copper anode X-
2 ray tube (1.5406 Å) and Kβ filter, at voltage equal to 40 kV and a current of 40 mA,
3 with speed of 200 seconds/step. Diffractograms were treated using OriginPro 8.5
4 software. Crystallite size was achieved using Scherrer equation:

$$5 \quad L_s = \frac{\kappa \times \lambda}{FWHM \times \cos \theta}$$

6 Where κ is a constant equal to 0.94; λ is the x-rays wave length equal to 0.154 nm;
7 FWHM is the full width at half maximum and θ in the Bragg angle in radian.

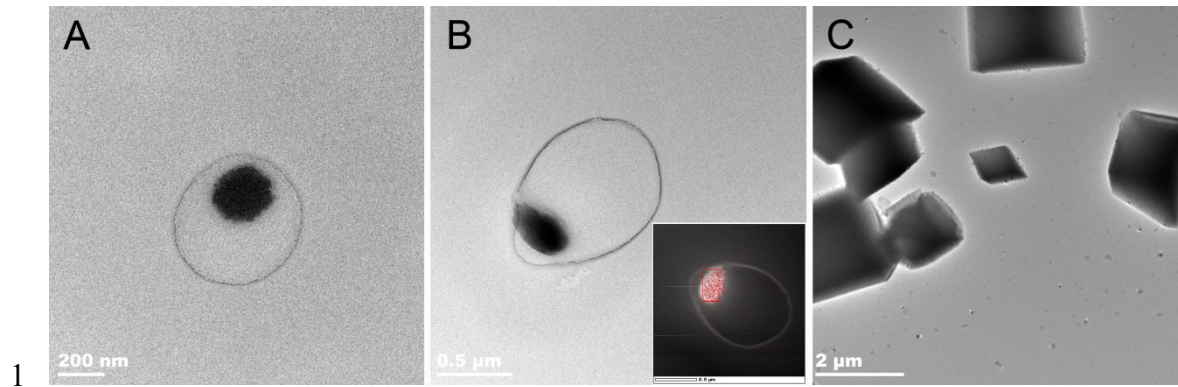
1 2. Supporting Figures



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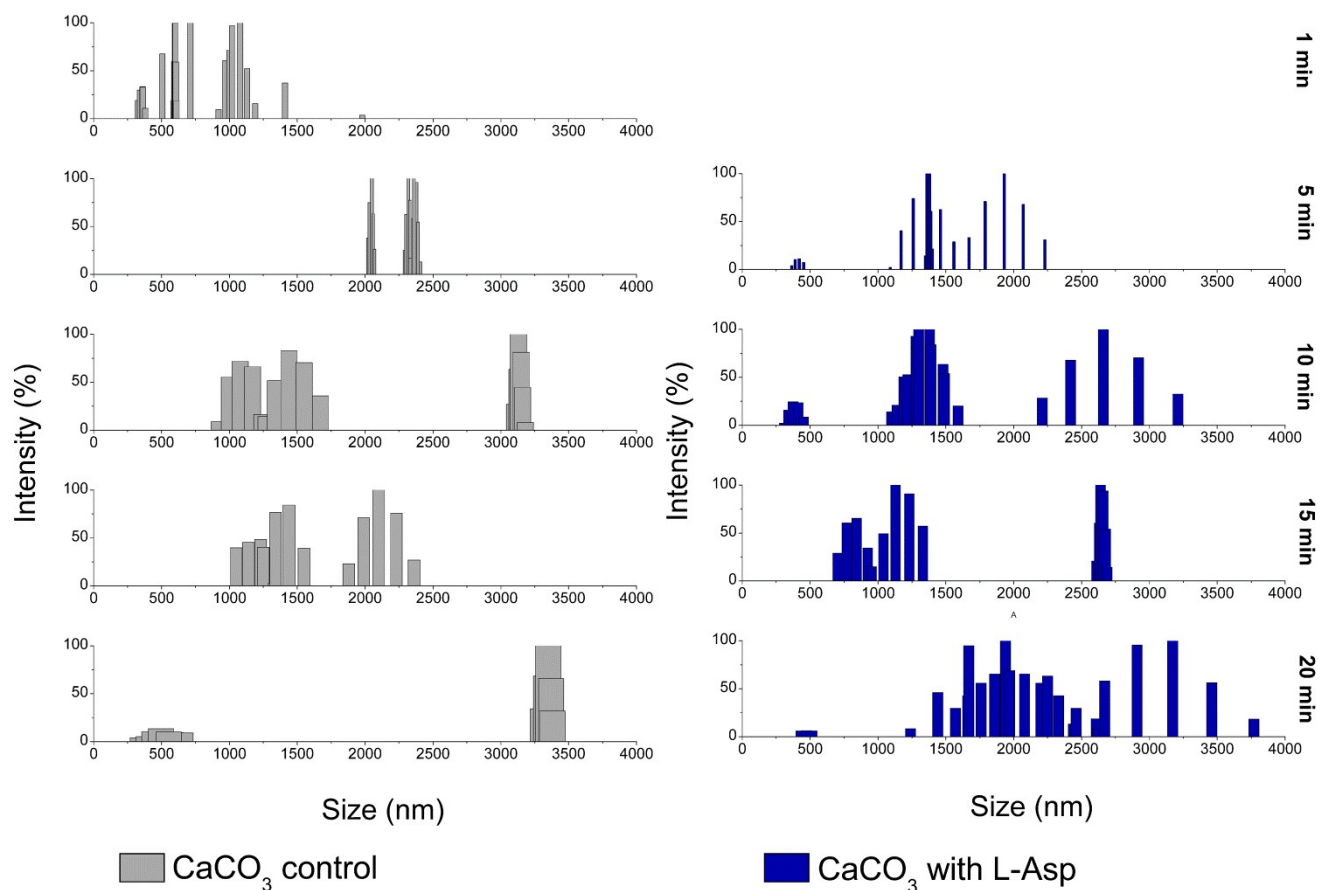
3 **Figure S1.** SEM images and EDS spectra of the *ex situ* experiments described on
4 section 1.1. A and B: CaCO₃ 100 mM; C and D: CaCO₃ 10 mM; E and F: CaCO₃ 5
5 mM. Mean size of crystals in A = 2.6 μm; C and E varying from 2.5 to 1.5 μm

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2 **Figure S2.** TEM images obtained from the *ex situ* experiments with interruption of the
3 reaction on the grid after 10 min. The presence of vesicle-like structures containing
4 electron-dense material can be observed (A and B). A calcium map obtained by STEM-
5 EDS is presented in the insert of (B), indicating that the vesicle-like structure is rich in
6 calcium. Some crystals already formed can be observed in (C). Note that even in figures
7 A and B, the straight segmented contours of the calcium rich regions suggest that at
8 least part of the growing structures are already crystalline.

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1 **Figure S3.** Intensity-based size distribution of the samples CaCO₃ control (grey) and
 2 CaCO₃ with L-Asp (blue) through time of reaction (1, 5, 10, 15 and 20 minutes).

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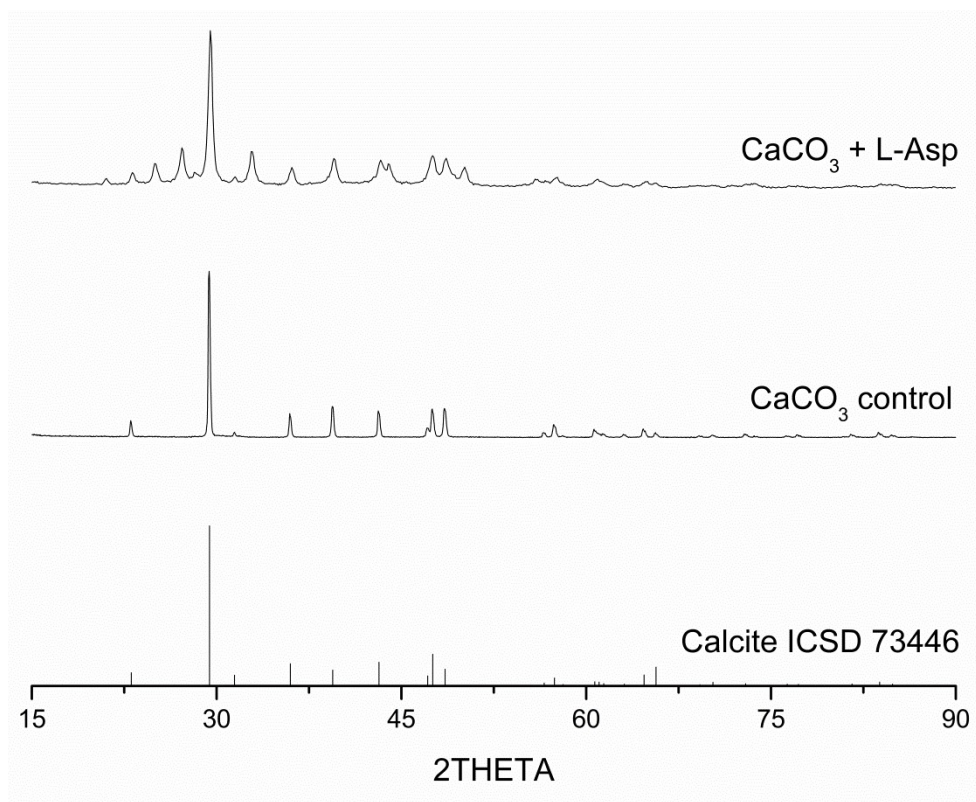
4 Table S1: Hydrodynamic diameter and polydispersity index of CaCO₃ control and

5 CaCO₃ with L-Asp experiments through time of reaction analysed by DLS.

	CaCO ₃ control		CaCO ₃ with L-Asp	
	D _H (nm)	PI	D _H (nm)	PI
1 min	689 ±59	0.09 ±0.05	---	---
5 min	2779 ±297	0.28 ±0.16	1440 ±162	0.26 ±0.04
10 min	4041 ±757	0.37 ±0.08	1647 ±190	0.29 ±0.09
15 min	4958 ±584	0.40 ±0.07	2356 ±400	0.37 ±0.05
20 min	9736 ±4790	0.54 ±0.04	3055 ±258	0.34 ±0.07

6 ± Standard deviation; D_H: hydrodynamic diameter; PI: polydispersity index. Result of
 7 CaCO₃ with L-Asp sample after 1 min analysis is not shown since the particle count rate
 8 was lower than required by the equipment.

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2 **Figure S4.** X-ray diffractograms of the control experiment, without L-Asp (CaCO₃
 3 control), and the L-Asp experiment (CaCO₃ + L-Asp) compared with Calcite pattern
 4 (ICSD 73446) described on item 1.2. Both experiments produced crystals with
 5 diffraction pattern corresponding to calcite.

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7 **Table S2:** Crystallite size and interplanar distance of the most intense peak of each
 8 diffractogram in the Figure S4

Sample	Size (nm)	Interplanar distance (Å)	h k l
Calcite ICSD 73446	----	3.035	1 0 4
CaCO ₃ control	46.27	3.038	1 0 4
CaCO ₃ + L-Asp	20.62	3.029	1 0 4

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1 **3. Supplementary videos**

2 **Video S1:** Dynamic interaction between grains visualized during the control experiment
3 (without L-Asp) in continuous flow mode; the video was collected at 3 frames per
4 second (fps) and played at 15 fps.

5 **Video S2:** Oriented attachment of small grains and nuclei fusion and growth visualized
6 during the control experiment (without L-Asp) in continuous flow mode; the video was
7 collected at 3 fps and played at 25 fps.

8 **Video S3:** Displacement of vesicle-like structure along the reaction medium collecting
9 electron-dense material visualized during the experiment with L-Asp in continuous flow
10 mode; the video was collected at 3 fps and played at 25 fps.

11 **Video S4:** Different behaviors of vesicle-like structures containing electron-dense
12 particles and their fusion along the reaction medium visualized during the experiment
13 with L-Asp in continuous flow mode; the video was collected at 3 fps and played at 30
14 fps.