

Supplementary information

Halochromic modulation of amorphous calcium carbonate crystallization driven by pH-responsive bioinspired pigments

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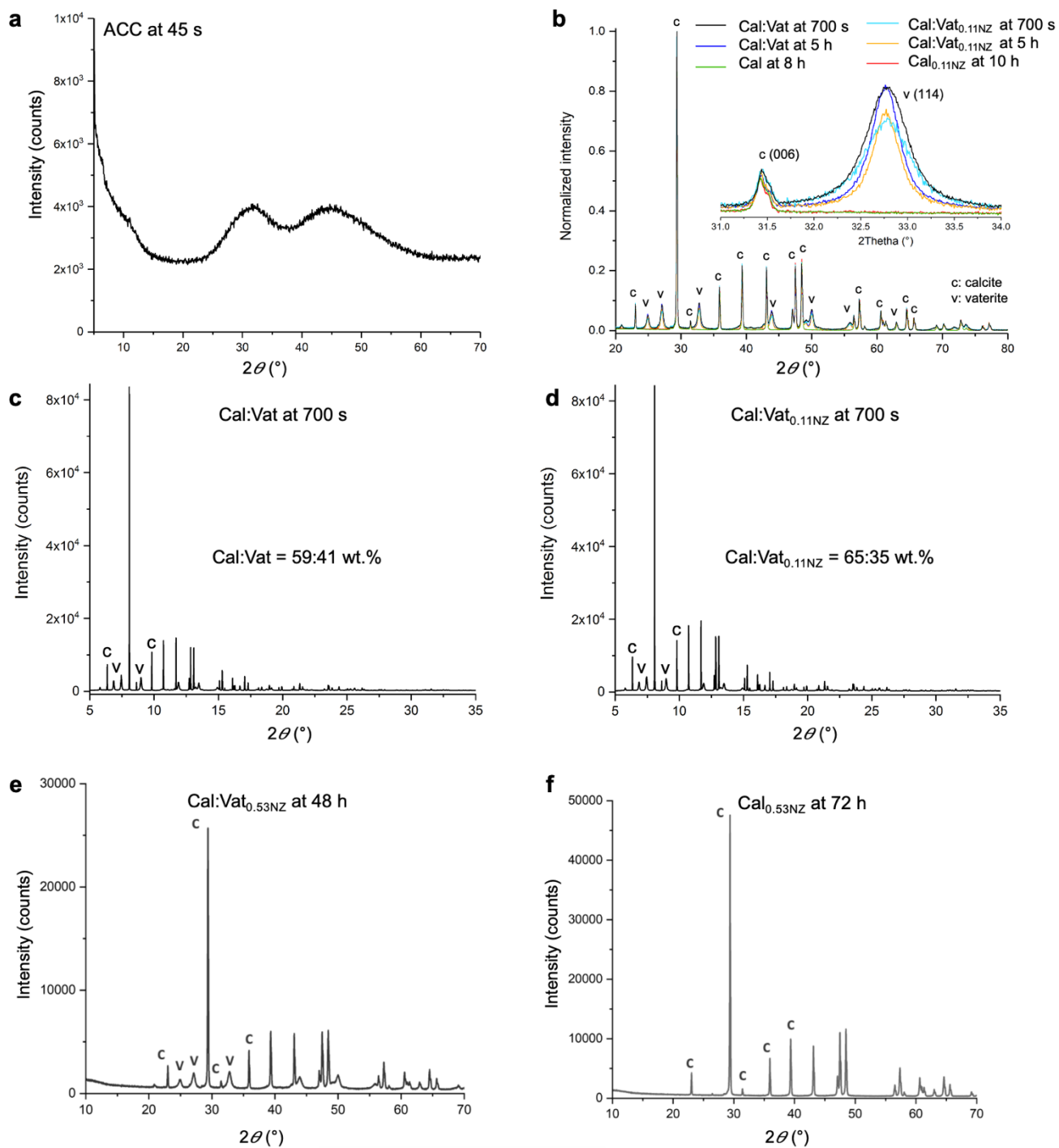
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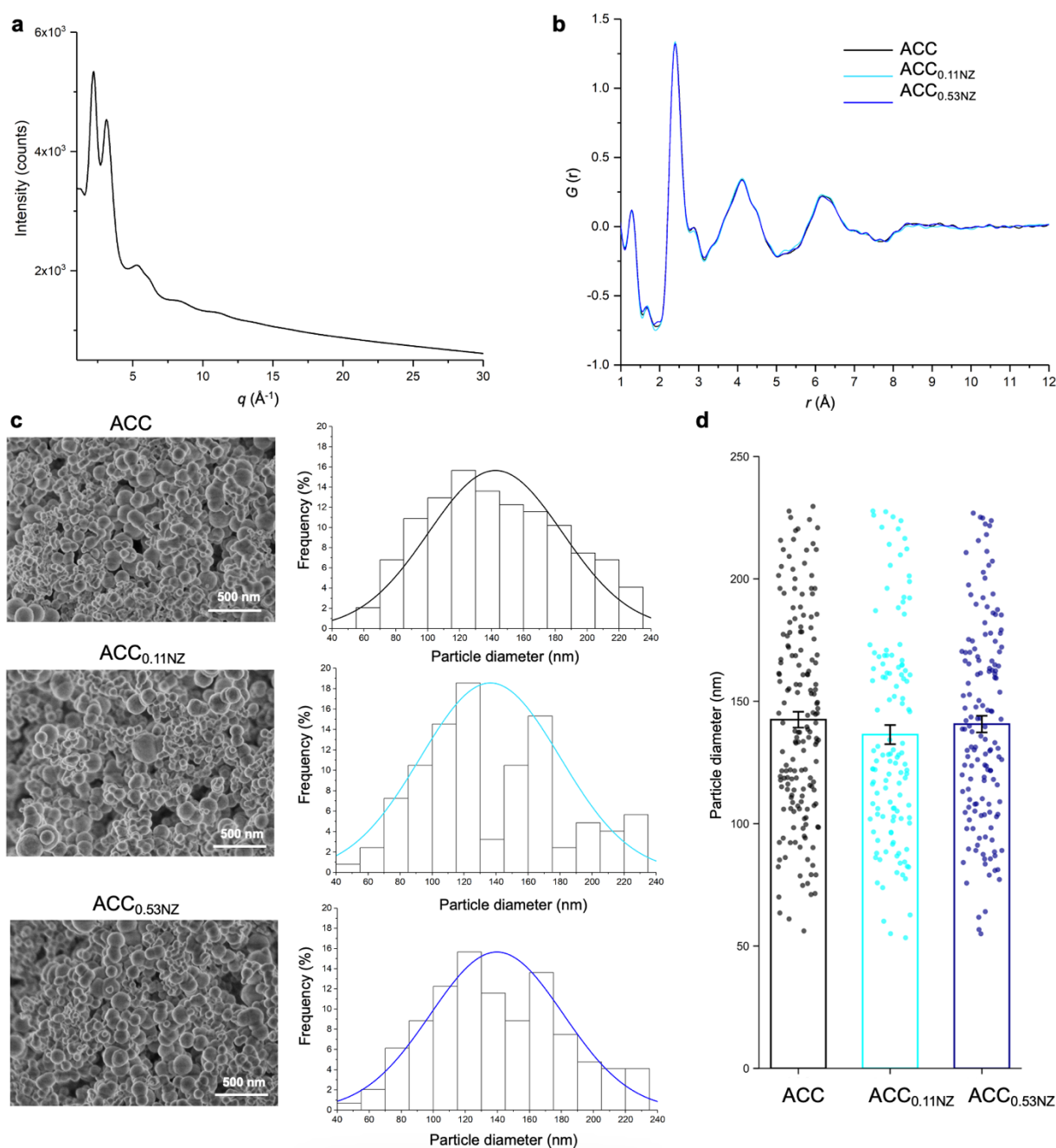
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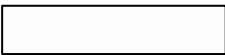


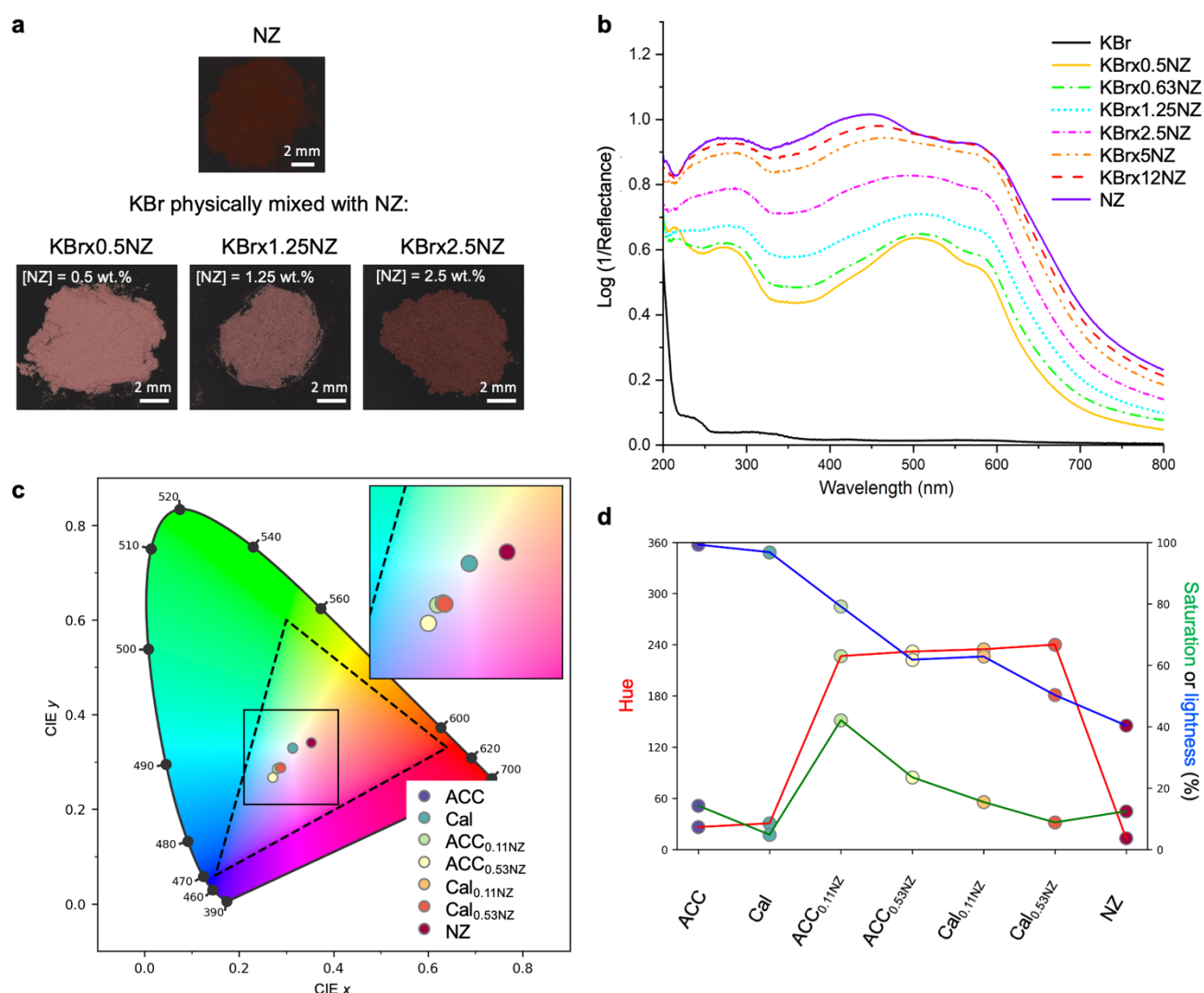
Supplementary Fig. 1. CaCO_3 polymorphs identification. Laboratory XRD profiles of **a)** ACC, **b)** Cal:Vat, Cal, Cal:Vat_{0.11NZ} and Cal_{0.11NZ} samples taken at different times of the reaction, **c-d)** synchrotron HR-XRD profiles of the Cal:Vat and Cal:Vat_{0.11NZ} samples at 700 s with the Cal:Vat ratio obtained from Rietveld refinements and **e-f)** laboratory XRD profiles of Cal:Vat_{0.53NZ}, showing a mixture of calcite and vaterite at 48 h and showing only calcite at 72 h.



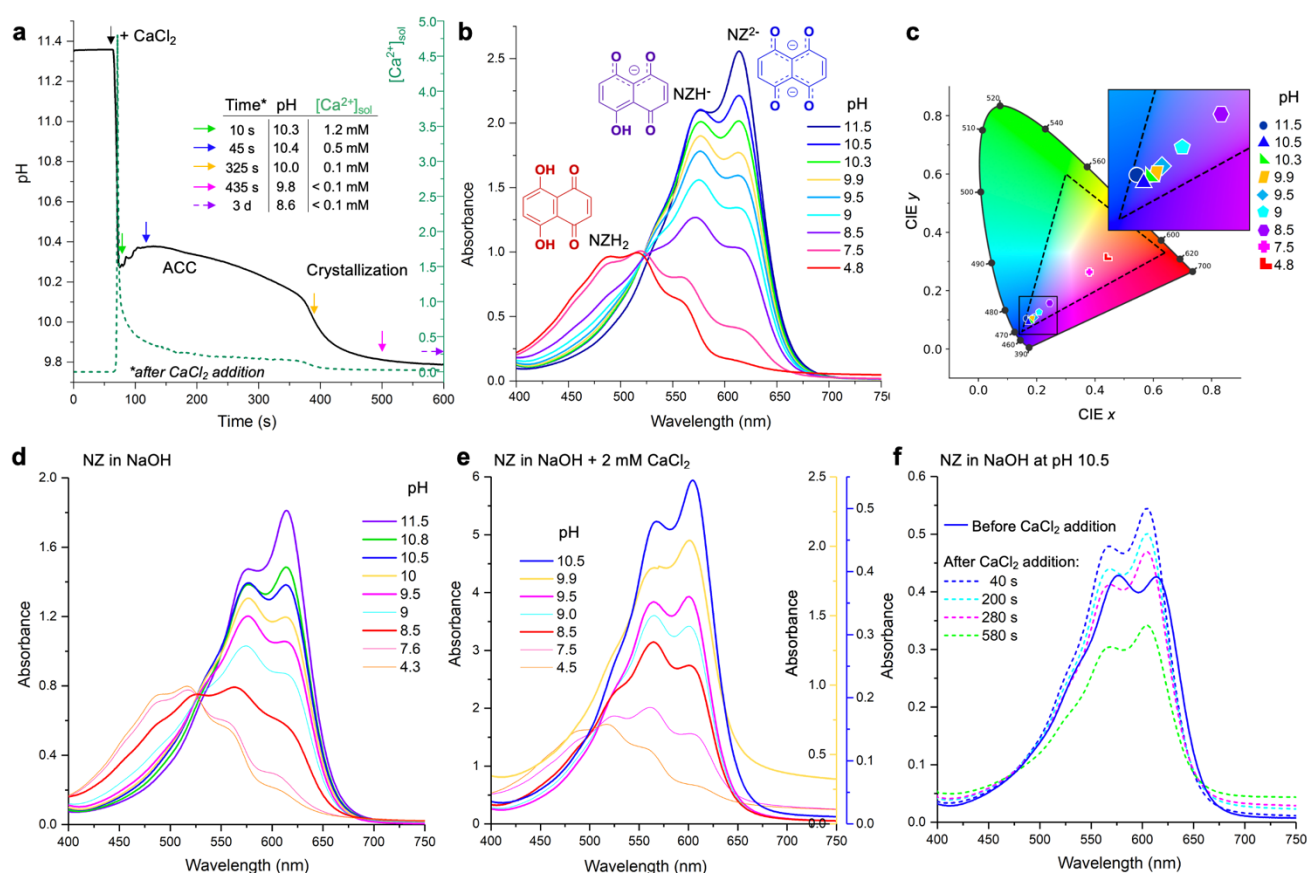
Supplementary Fig. 2. ACC characterization. **a)** XRD profile of ACC acquired at the synchrotron ID15A beamline (ESRF, France), **b)** PDF analysis of ACC (black), ACC_{0.11}NZ (cyan) and ACC_{0.53}NZ (blue) calculated from ID15 data that all show the same characteristic inter-atomic distances with positions at 2.4, 2.9, 4.1 and 6.2 $\text{\AA}$, containing respectively the contributions from Ca-O pairs, O-O pairs, Ca-O/Ca-Ca pairs (1st shell) and Ca-O/Ca-Ca pairs (2nd shell) and exhibiting short and medium-range order up to 10 $\text{\AA}$, **c)** characteristic SEM micrographs of ACC, ACC_{0.11}NZ and ACC_{0.53}NZ with their respective nanoparticle size distributions plotted in the forms of histograms and gaussian functions and **d)** bar chart with the corresponding data points as dot plots for ACC ($n = 168$), ACC_{0.11}NZ ($n = 124$) and ACC_{0.53}NZ ($n = 146$), the error bar correspond to the standard error of the mean.

Supplementary Table 1. L^* , a^* , b^* values calculated from the UV-visible diffuse reflectance spectra as well as sRGB values and simulated colors calculated with the color convertor of the nixsensor.com website.

Samples	L^* , a^* , b^*	sRGB values	Simulated colors
ACC	99.3, 0.1, 0.2	253 ,253, 253	
ACC _{0.11NZ}	76.9, 3.9, -18.6	179, 189, 224	
ACC _{0.53NZ}	59.3, 6.8, -21.6	135, 141, 180	
Cal	97.2, 0.1, 0.2	247, 247, 247	
Cal _{0.11NZ}	62.0, 4.7, -14.3	146, 148, 175	
Cal _{0.53NZ}	49.9, 4.9, -12.2	117 ,117, 139	



Supplementary Fig. 3. Color quantification of all samples. **a)** Stereo microscope images of NZ powder and powders obtained by physical mixing of KBr and NZ in different amounts from 0.5 to 12 wt.%, and **b)** corresponding UV-visible spectra acquired by DRS measurements, **c)** CIE 1931 chromaticity diagram of NZ, ACC, ACC_{0.11}NZ, ACC_{0.53}NZ, Cal_{0.11}NZ, and Cal_{0.53}NZ showing the respective hues with **d)** the saturation and lightness parameters that better allow for distinguishing the different colors of the samples.



Supplementary Fig. 4. UV-vis in solution. **a)** pH and free $[Ca^{2+}]$ profiles during ACC precipitation and crystallization in the presence of 0.53 mM NZ, pH and $[Ca^{2+}]$ values at characteristic time points are summarized in the table (insert), **b)** UV-visible spectra of 40 mM Na_2CO_3 solutions with 0.53 mM NZ at pH from 11.5 to 4.8 adjusted with 0.1 M HCl, **c)** CIE 1931 diagram obtained based on the spectra shown in b) and UV-visible spectra of 40 mM NaOH solutions at pH from 11.5 to ≈ 4.5 adjusted with 0.1 M HCl with **d)** 0.53 mM NZ without Ca and **e)** 0.11 or 0.53 mM NZ with 2 mM $CaCl_2$, and **f)** of a 40 mM NaOH solution with 0.11 mM NZ at pH 10.5 before and after the addition of 2 mM $CaCl_2$ from 40 s to 580 s.

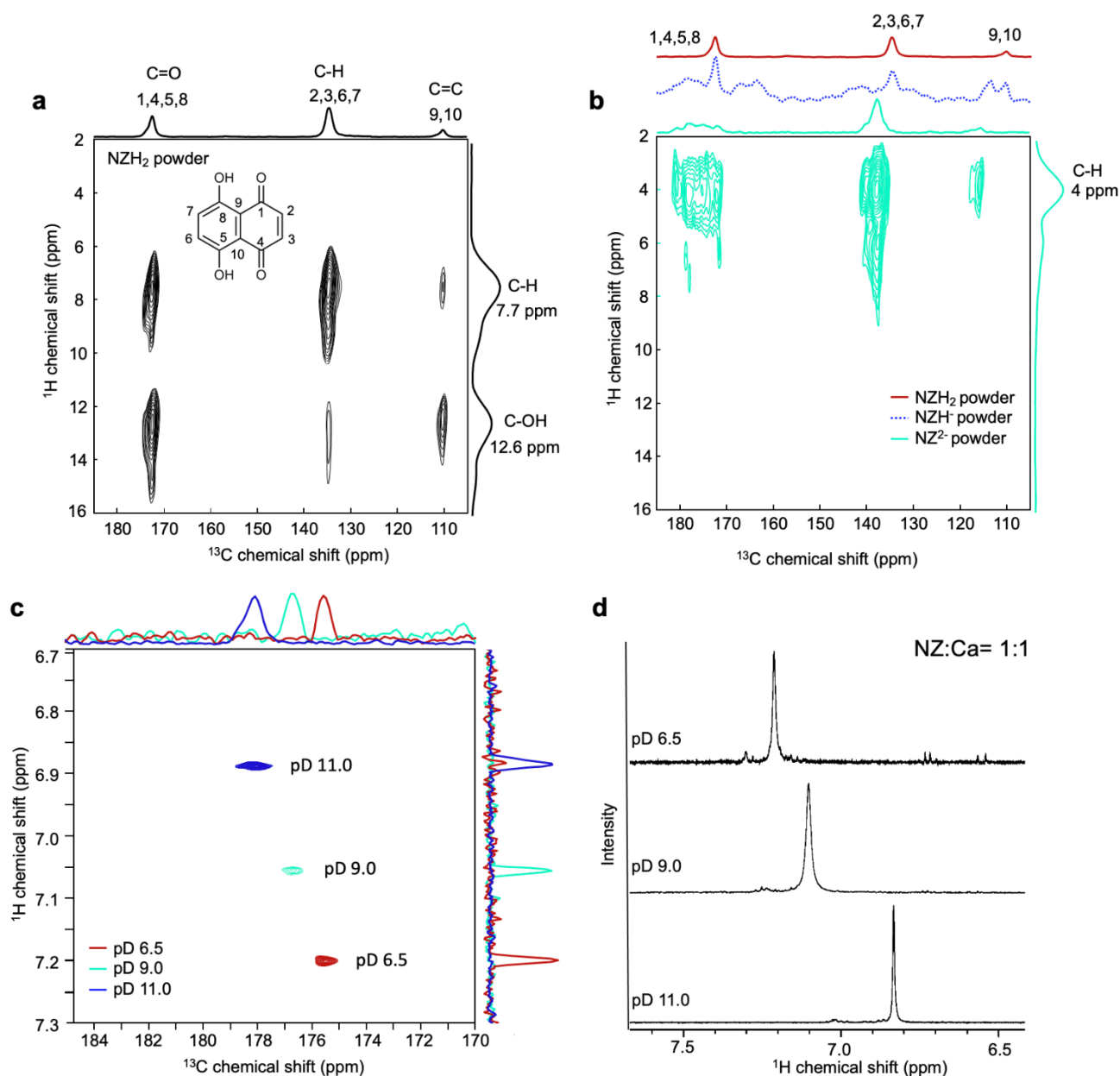
Supplementary Note 1

Supplementary Fig. 4b illustrates the progressive changes in the intensities of the UV-visible spectral bands as the pH increases. At pH 4.8 and pH 7.5, the solution is red, and the 515 nm band, attributed to the NZH_2 form, is the most intense. At pH 8.5, the solution is violet and the 575 nm band that corresponds to the NZH^- form is the most intense whereas at pH 10.3, the solution turns violet-blue and the band at 575 nm shows equal intensity to the one at 615 nm, attributed to the NZ^{2-} form, and thus indicating the presence of both NZH^- and NZ^{2-} forms. Finally, at pH 11.5, the 615 nm band is the most intense, and the solution is deep blue, due to the presence of NZ^{2-} only. We note the significant

decrease in the extinction coefficient with decreasing pH, likely due to the aggregation of the NZ molecules ¹, which are less soluble at acidic pH (0.06 mM at pH 5.5) than at basic pH (2.2 mM at pH 11.5).

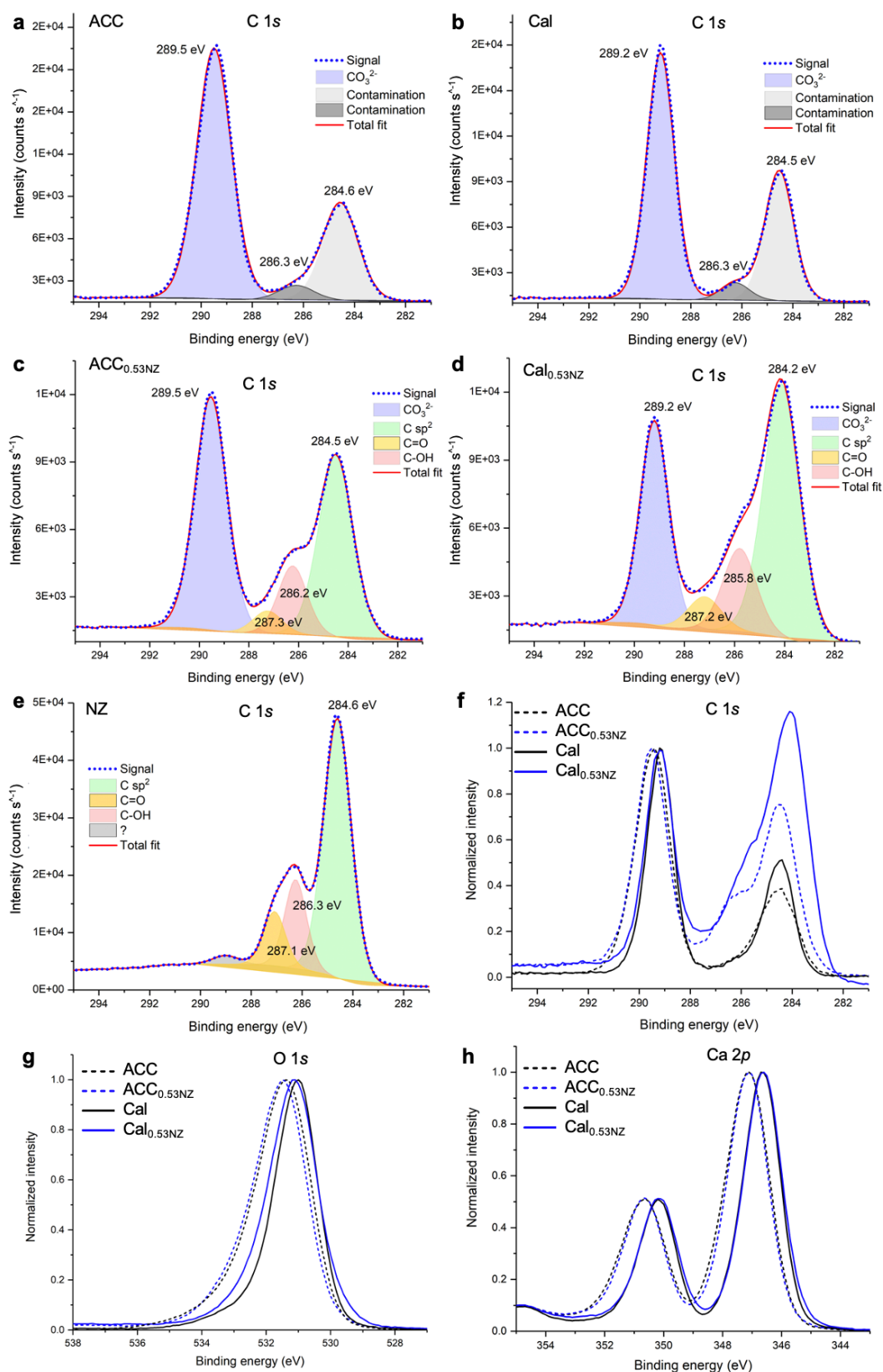
Supplementary Fig. 4d and 4e display the UV-Vis spectra of NZ measured immediately after the addition of 2 mM Ca^{2+} . They show the characteristic NZ absorption bands described above. However, the relative intensity of those bands, depending on pH values, differs from that in the absence of Ca^{2+} . This results in a decrease in the pK_a values by one pH unit. Indeed, pK_a values extracted by fitting the absorbance spectra acquired using equation 1 yielded to $\text{pK}_{a1} = 7.5$ and $\text{pK}_{a2} = 9.4$.

Finally, the spectra measured at pH 10.5 as a function of time until 580 s after Ca^{2+} addition (Supplementary Fig. 4f), exhibit the characteristic NZ absorbance bands whose intensities only decrease slightly, indicating no significant oxidative decomposition of NZ in these conditions.



Supplementary Fig. 5. NMR data of the reference samples. 2D ^1H - ^{13}C HETCOR solid-state NMR spectrum of **a)** NZ powder in its protonated form (NZH_2) that shows the correlations between the three carbon resonances at 173 ppm for C=O, 135 ppm for =C-H and 110 ppm for C=C with the two proton resonances at 7.7 ppm for =C-H and 12.6 ppm for C-OH and **b)** NZ prepared at pH 12 (NZ^{2-} powder) that shows a shift to lower ppm values of the ^1H signals and the decrease of the C-OH resonance with increasing pH. The ^{13}C CPMAS NMR spectra of NZH_2 powder, and NZ prepared at pH 9 (NZH^- powder) are also shown, **c)** ^1H - ^{13}C 2D HMBC liquid-state NMR spectra of three NZ solutions prepared in D_2O at pD = 6.5 (gold), 9 (red) and 11 (blue) measured at 25 °C for pD = 11 and 37 °C for pD = 6.5 and 9.0 to increase NZ solubility. The ^{13}C resonance corresponding to the 1,4,5,8 carbons, shifts toward higher ppm values with pH increase (from 175.6 ppm to 176.4 ppm and to 178.2 ppm)

whereas the ^1H resonance to lower ppm values (from 7.2 ppm to 7.06 ppm to 6.89 ppm) and **d)** ^1H spectra of the three solutions of c) in the presence of Ca^{2+} ions (NZ:Ca, 1:1), which exhibit smaller ^1H peak shifts than the ones evidenced upon pH variations. This suggests that peak shifts observed for $\text{Ca}_{0.53}\text{NZ}$ (Fig. 2d) are more likely due to NZ deprotonation rather than to Ca complexation. Chelation of metallic ions with anthraquinone dyes was previously reported to also induce ^{13}C peak shifts ^{2,3}.



Supplementary Fig. 6. XPS data. XPS spectra of C 1s and fitted components (CO_3^{2-} , C sp^2 , C-OH and C=O) of **a)** ACC, **b)** Cal, **c)** ACC_{0.53}NZ, **d)** Cal_{0.53}NZ, **e)** NZ, the peaks at 284.5/6 $\pm$ 0.1 and 286.3 $\pm$ 0.1 eV for Cal and ACC are due to the presence of carbonaceous contamination, the peaks at 287.3 $\pm$ 0.1 and

287.2±0.1 eV, 286.2±0.1 and 285.8±0.1 eV as well as 284.4±0.2 eV and 284.2±0.2 eV for ACC_{0.53NZ} and Cal_{0.53NZ} are respectively attributed to the C=O bonds, C-OH bonds and the C *sp*² bonds of the NZ molecules, **f)** C 1s, **g)** O 1s and **h)** Ca 2*p* core levels of ACC, ACC_{0.53NZ}, Cal and Cal_{0.53NZ} samples. Notably, the carbonate contributions on the C 1s, O 1s and Ca 2*p* core levels of ACC exhibit higher binding energies than those of calcite (289.2±0.1 eV) and are closer to that of aragonite ⁴.

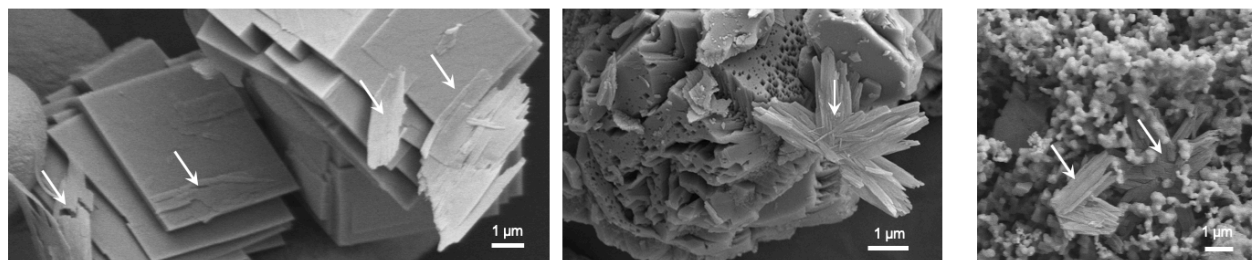
Supplementary Table 2. NZ content in solution before ACC precipitation as well as in the solid amorphous and calcite samples ACC_{0.11NZ}, ACC_{0.53NZ}, Cal_{0.11NZ} and Cal_{0.53NZ} (before and after bleaching). NZ contents of the powders are obtained from UV visible measurements of the solution after the dissolution of the powders. Surface atomic percentages ($C_{\text{organic}}/C_{\text{organic+inorganic}}$) are obtained from XPS analysis.

Samples		ACC _{0.11NZ}	ACC _{0.53NZ}	Cal _{0.11NZ}	Cal _{0.53NZ}	Bleached	
						Cal _{0.11NZ}	Cal _{0.53NZ}
NZ content							
Initial [NZ] in Na ₂ CO ₃ solution (mM)		0.11	0.53	0.11	0.53	0.11	0.53
Initial NZ/Ca (%)		0.28	1.33	0.28	1.33	0.28	1.33
Amount of dissolved sample (mg)		60	20	50	20	100	50
[Ca ²⁺] after CaCO ₃ dissolution (mM)		12	4	10	4	20	12.5
[NZ] after CaCO ₃ dissolution (mM)*		0.033	0.051	0.026	0.048	0.006	0.008
[NZ] associated with CaCO ₃ *	mM	0.109	0.51	0.10	0.48	0.01	0.03
	mol.%	0.27	1.28	0.25	1.2	0.03	0.08
	wt.%	0.51	2.40	0.47	2.26	0.05	0.15
Surface atomic percentage $C_{\text{organic}}/C_{\text{organic+inorganic}}$ (%)**		-	54	-	69	-	-

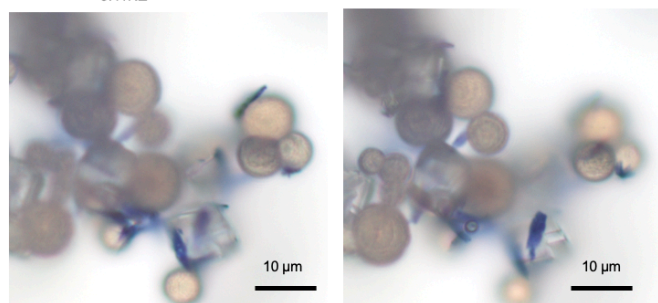
*Obtained from UV-visible measurements

**Surface atomic percentage obtained from XPS analysis, for pure ACC: 31% and for pure calcite: 38%.

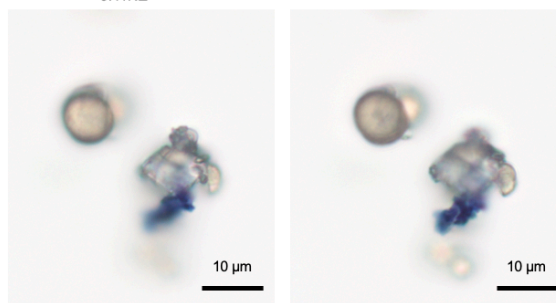
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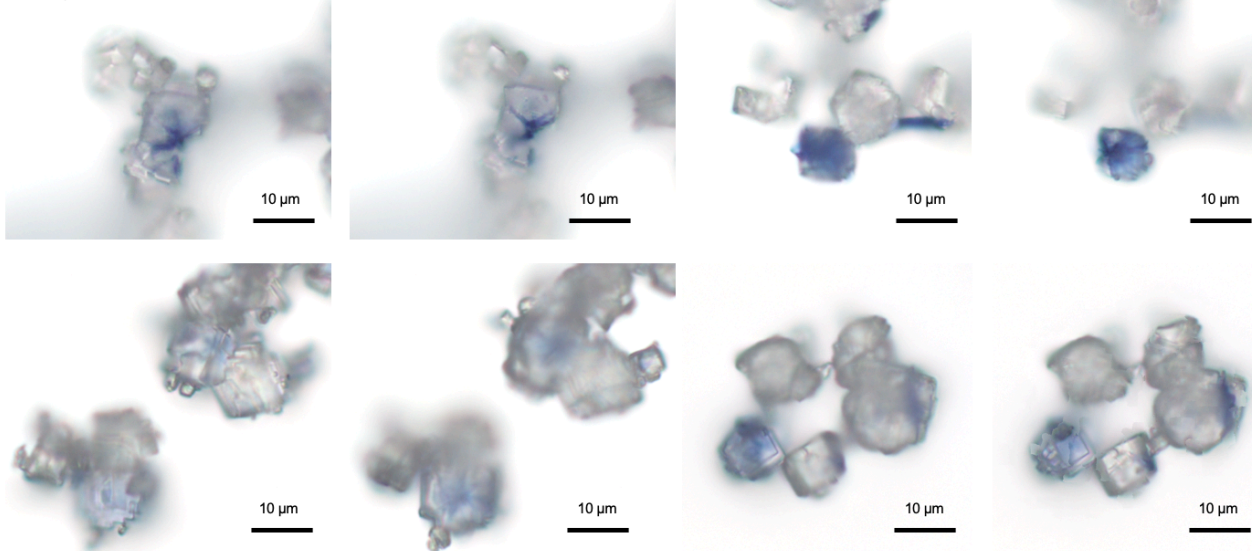
b Cal:Vat_{0.11}NZ at 700 s



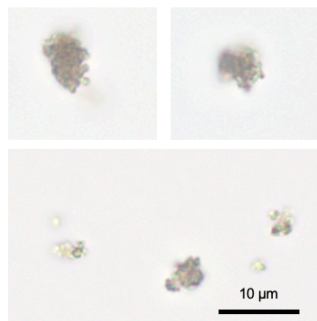
c Cal:Vat_{0.11}NZ at 2.5 h



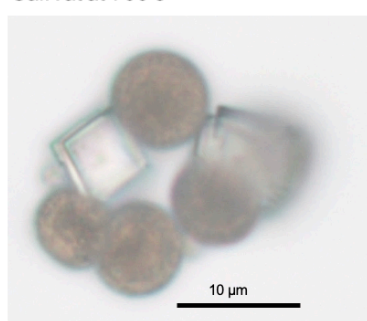
d Cal_{0.11}NZ at 18 h



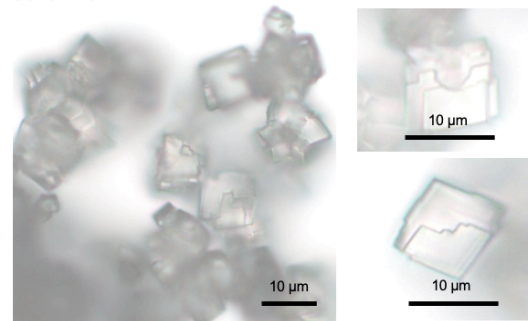
e ACC at 45 s



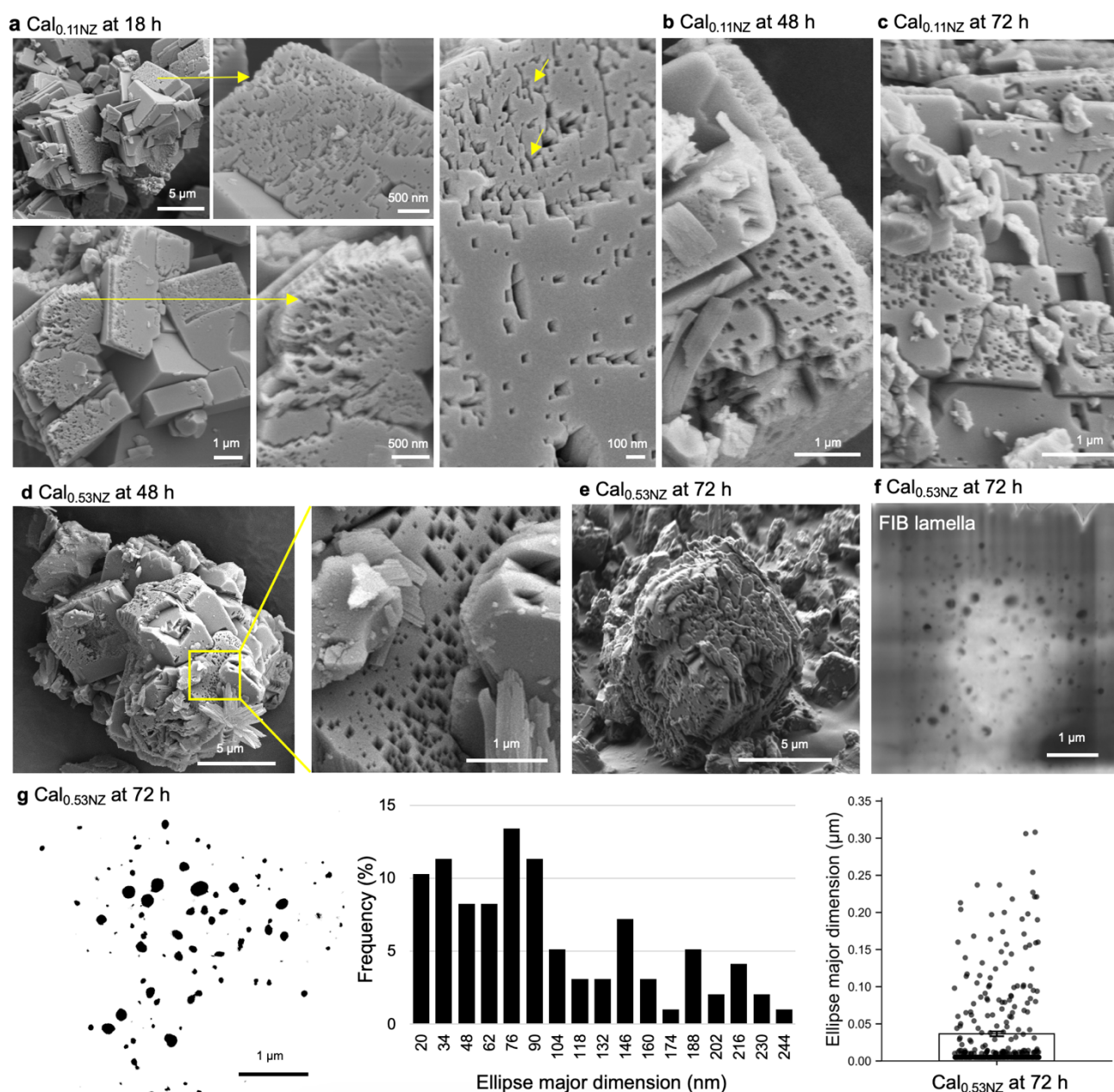
Cal:Vat at 700 s



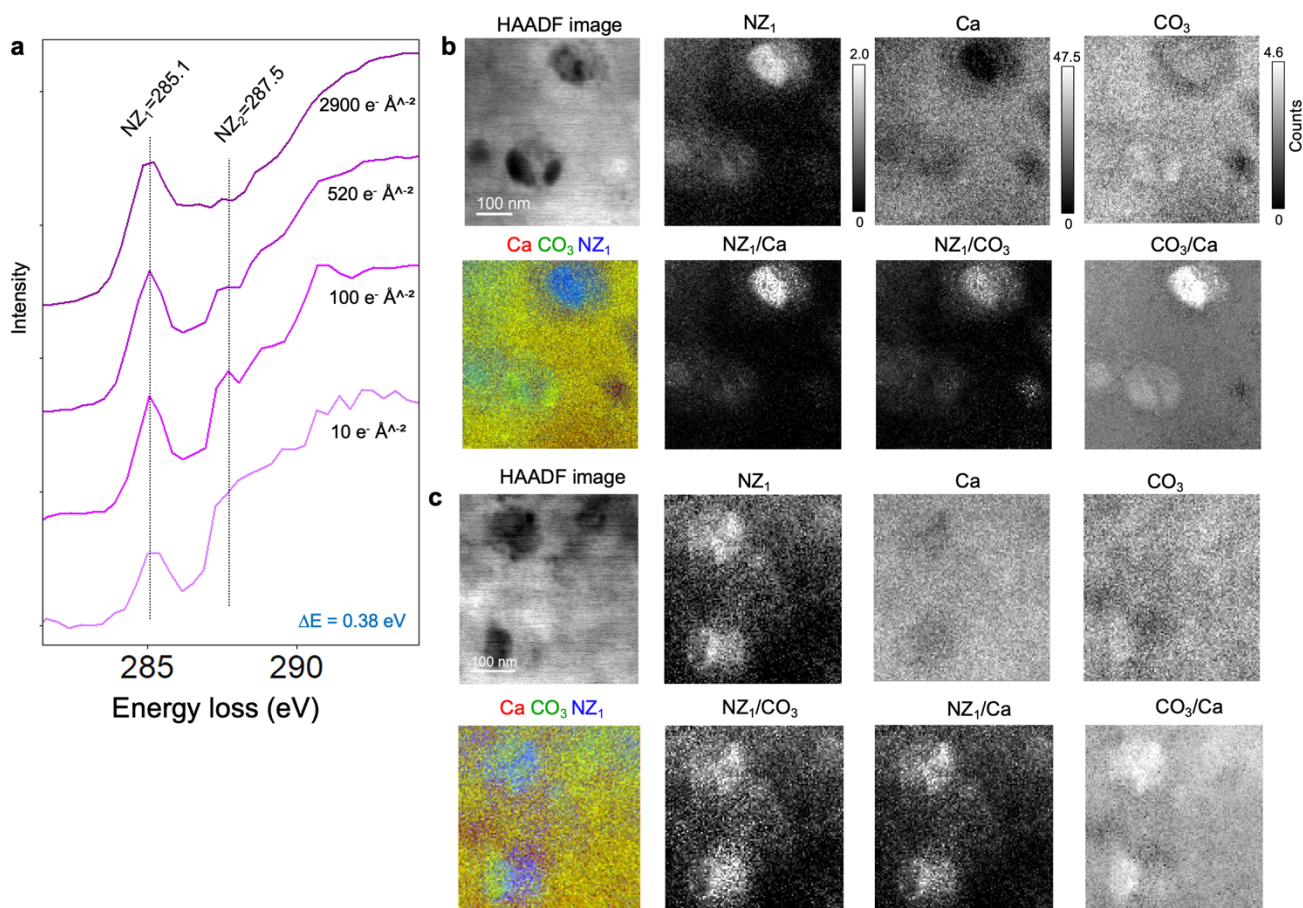
Cal at 18 h



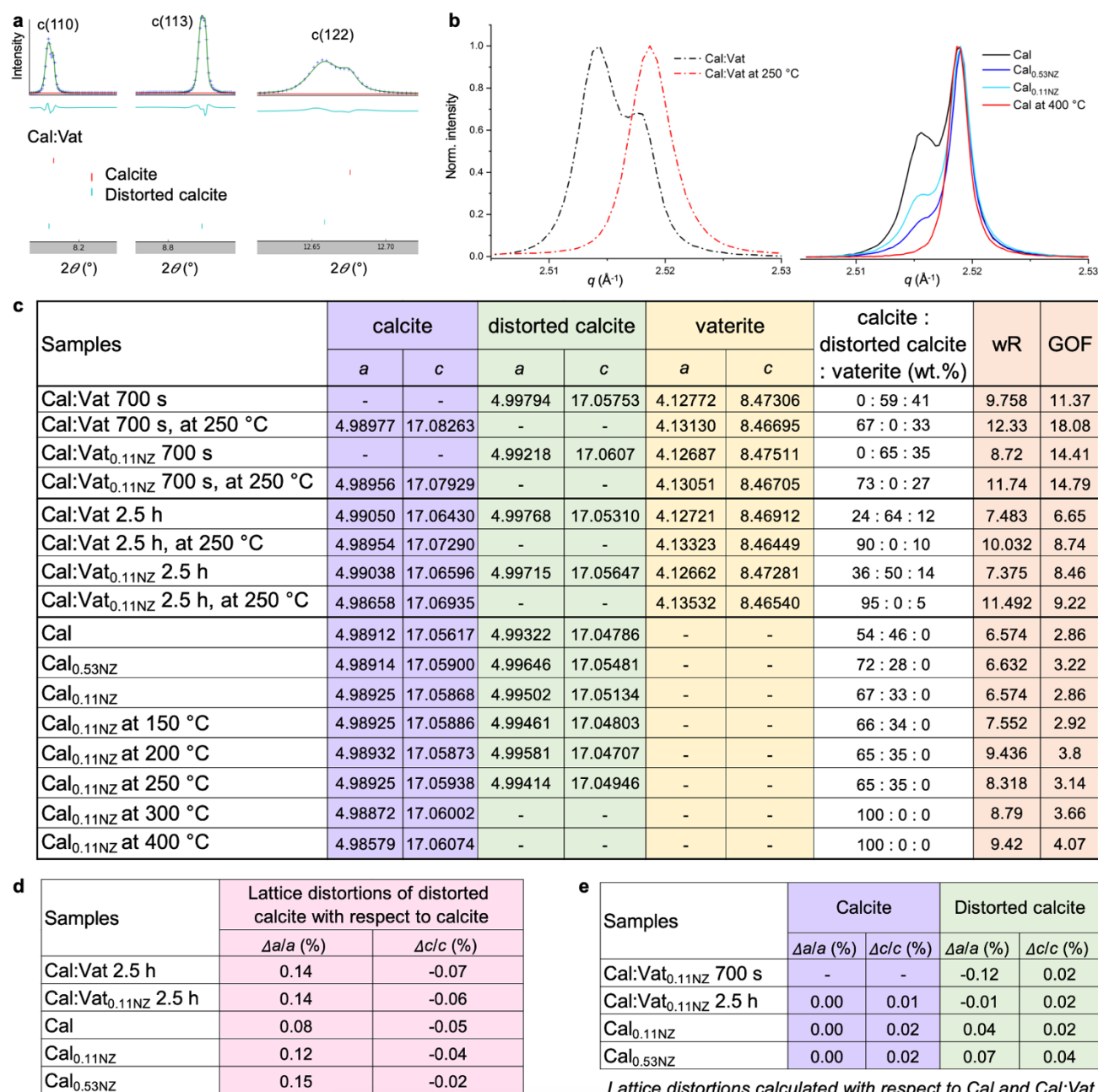
Supplementary Fig. 7. NZ visualization. a) FEG-SEM observations of crystallized NZ (indicated by the white arrows) at the surface of calcite crystals and ACC nanoparticles, transmission optical microscope images with two different focal planes of **b)** Cal:Vat_{0.11}NZ at 700s, **c)** Cal:Vat_{0.11}NZ at 2.5h, **d)** Cal_{0.11}NZ at 18h and **e)** transmission optical microscope images of ACC, Cal:Vat and Cal samples.



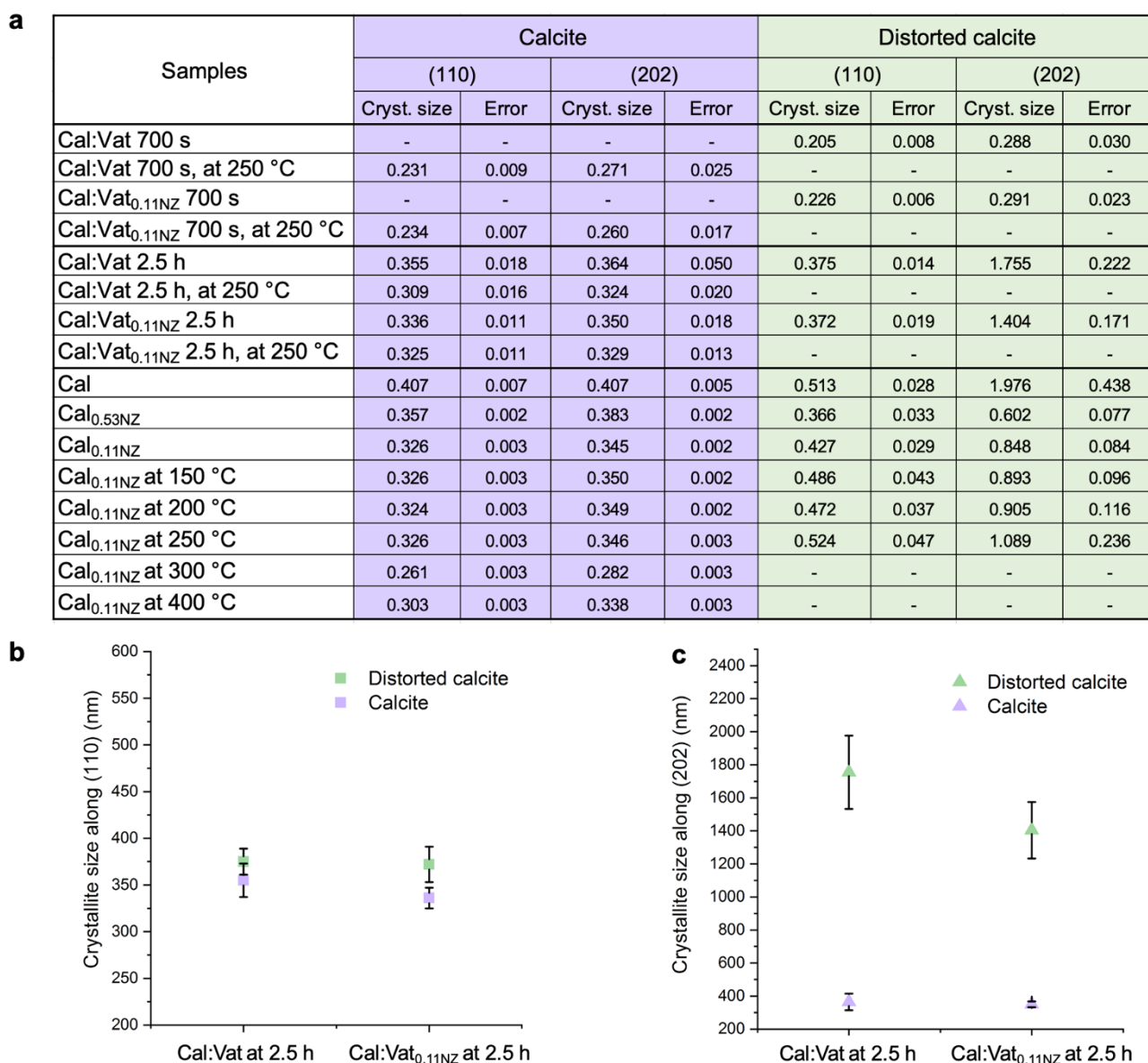
Supplementary Fig. 8. External and internal pores analyses. SEM micrographs of **a)** $\text{Cal}_{0.11\text{NZ}}$ after 18 h synthesis, the yellow arrows show crevasses at the surface of the crystals, **b-c)** $\text{Cal}_{0.11\text{NZ}}$ after 48 and 72 h synthesis, **d)** $\text{Cal}_{0.53\text{NZ}}$ after 48. h synthesis. At $[\text{NZ}] = 0.53 \text{ mM}$, the rhombohedral pits are similar to the ones at $[\text{NZ}] = 0.11 \text{ mM}$ in terms of shape and size but the overall calcite crystal shape is less rhombohedral and more isotropic with no defined facets, **e)** selected crystal of a bleached $\text{Cal}_{0.53\text{NZ}}$ sample after 72 h synthesis with **f)** its FIB lamella observed under SEM (in lens detector) and **g)** particle analysis of the internal pores with $n=334$ particles.



Supplementary Fig. 9. EELS data. **a)** C K-edge EELS spectra from NZ powder measured at a low energy dispersion ΔE of 0.38 eV per channel, required for simultaneous C and Ca analysis, and electron doses from 10 to 2900 e⁻ Å⁻² averaged over an area of 500 x 500 nm². Two peaks are detected at 285.1 eV and 287.5 eV denoted NZ₁ and NZ₂, respectively. As the dose increases (≥ 100 e⁻ Å⁻²), NZ degrades progressively, with peak broadening of the NZ₁ peak and the emergence of peak NZ₂, attributed to CO formation from NZ radiolysis⁵. At the lowest dose (10 e⁻ Å⁻²), spectral features remain undegraded⁶, but the large pixel size (10 nm) and the low signal-to-noise ratio prevent chemical mapping. Therefore, the NZ₁ peak is used here to detect and map NZ because it dominates the NZ spectrum for all explored doses. In addition, the sample being a FIB lamella with NZ as the sole organic species, NZ₁ mapping is unambiguous and free from carbon film contributions. **b-c)** HAADF images of two selected areas from the FIB lamella with their corresponding EELS maps obtained by integrating the C K-edge of the NZ₁ peak, the Ca L_{2,3}-edge and the C K-edge of the CO₃ peak. The corresponding RGB composite images combining the spatial distributions of Ca (in red), CO₃ (in green) and NZ₁ (in blue) and the NZ₁/Ca, NZ₁/CO₃ and the CO₃/Ca maps are shown.



Supplementary Fig. 10. Rietveld refinement analysis. **a)** fits of the HR-XRD profile for the Cal:Vat sample taken at 2.5 h of the reaction, **b)** (110) HR-XRD reflections of the calcite phase of Cal:Vat, Cal:Vat_{0.11NZ} at 2.5 h before and after annealing at 250 °C, Cal before and after annealing at 400 °C as well as Cal_{0.11NZ} and Cal_{0.53NZ}, **c)** results of Rietveld refinement of all samples, **d)** lattice distortions of the distorted nanodomains calculated with respect to the undistorted calcite nanodomains and **e)** lattice distortions of the calcite and distorted nanodomains calculated with respect to Cal:Vat and Cal.



Supplementary Fig. 11. Crystallite size analyses. **a)** Crystallite sizes (μm) and error (μm) of the calcite and distorted calcite nanodomains along the (110) and (202) directions obtained after peak fitting using a Voigt function according to Pokroy and Zolotoyabko, 2006⁷ and crystallite sizes of the calcite and distorted calcite nanodomains along **b)** the (110) and **c)** the (202) directions of the Cal:Vat and Cal:Vat_{0.11NZ} samples at 2.5h. The error bars correspond to the standard error of the fitting with the Voigt function.

Supplementary references

1. Savko, M. *et al.* Ground and excited state properties of naphthazarin: Absorption spectroscopy and theoretical modeling study. *J. Mol. Struct.-Theochem* **803**, 79–87 (2007).

2. Cavalcanti, G. R. S. *et al.* Inorganic-organic hybrid pigments based on carminic acid and clay minerals. *Dyes Pigments* **190**, 109306 (2021).
3. Trigueiro, P. *et al.* When anthraquinone dyes meet pillared montmorillonite: Stability or fading upon exposure to light? *Dyes Pigments* **159**, 384–394 (2018).
4. Chu, D. H. *et al.* CO₂ mineralization into different polymorphs of CaCO₃ using an aqueous-CO₂ system. *RSC Adv.* **3**, 21722–21729 (2013).
5. Chaupard, M. Electron spectromicroscopy : from organic-inorganic drug nanocarriers to biological systems. (Université Paris-Saclay, 2023).
6. Chaupard, M. *et al.* Nanoscale Multimodal Analysis of Sensitive Nanomaterials by Monochromated STEM-EELS in Low-Dose and Cryogenic Conditions. *ACS Nano* **17**, 3452–3464 (2023).
7. Pokroy, B., Fitch, A. & Zolotoyabko, E. The Microstructure of Biogenic Calcite: A View by High-Resolution Synchrotron Powder Diffraction. *Adv. Mater.* **18**, 2363–2368 (2006).