membranes

MDPI

Supporting Information

Influence of Intramineral Proteins on the Growth of Carbonate Crystals Using as a Scaffold Membranes of Ratite Birds and Crocodiles Eggshells

Nerith R. Elejalde-Cadena [1,2], Denisse Hernández [2], Francesco Capitelli [3], Selene R. Islas [4], Maria J. Rosales-Hoz [5], Michele Zema [6,7], Serena C. Tarantino [8,9], Dritan Siliqi [7,*] and Abel Moreno [2,*]

[1] Institute of Physics, National Autonomous University of Mexico, Circuito de la Investigación Científica s/n, Ciudad Universitaria, Ciudad de Mexico 045010, Mexico; rocioec@fisica.unam.mx
[2] Institute of Chemistry, National Autonomous University of Mexico, Av. Universidad 3000, Ciudad de Mexico 04510, Mexico; carcamo@unam.mx (A.M); 316298290@quimica.unam.mx (D.H.)
[3] Institute of Crystallography (IC), National Research Council (CNR), Via Salaria km 29,300, 00016 Rome, Italy; francesco.capitelli@ic.cnr.it
[4] Instituto de Ciencias Aplicadas y Tecnología, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Cd. Universitaria, Ciudad de Mexico 045010, Mexico; selene.islas@icat.unam.mx
[5] Departamento de Química, Centro de Investigación y de Estudios Avanzados, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Ciudad de Mexico 07360, Mexico; mrosales@cinvestav.mx
[6] Department of Earth and Geoenvironmental Sciences, University of Bari "Aldo Moro", Via E. Orabona 4, 70125 Bari, Italy; michele.zema@uniba.it
[7] Institute of Crystallography (IC), National Research Council (CNR), Via Amendola 122/O, 70126 Bari, Italy
[8] Department of Chemistry, University of Pavia, Vialle Taramelli 16, 27100 Pavia, Italy; serenachiara.tarantino@unipv.it
[9] Institute of Geoscience and Georesources (IGG), National Research Council (CNR), Via Ferrata 1, 27100 Pavia, Italy
* Correspondence: dritan.siliqi@ic.cnr.it (D.S.); carcamo@unam.mx (A.M.)

Keywords: Membrane; Eggshell; Biomineralization; Intramineral proteins; Biocalcification; Biosilicification; Biomorphs.

Supplementary information includes the following information:

1. Identification of Intramineral Proteins by SDS-PAGE

Table S1. Fractions collected from injections made with 50 mM sodium citrate + 150 mM NaOH pH 4.0 at a flow rate of 0.5 mL/min. Fractions were collected every 1 mL and the volume in mL of the collected fractions is given. **Fxn:** Fraction.

Fxn	1	2	3	4	5	6
Ostrich	17.5	18	18.5	19	19.5	20
Emu	19	19.5	20	20.5	21	21.5

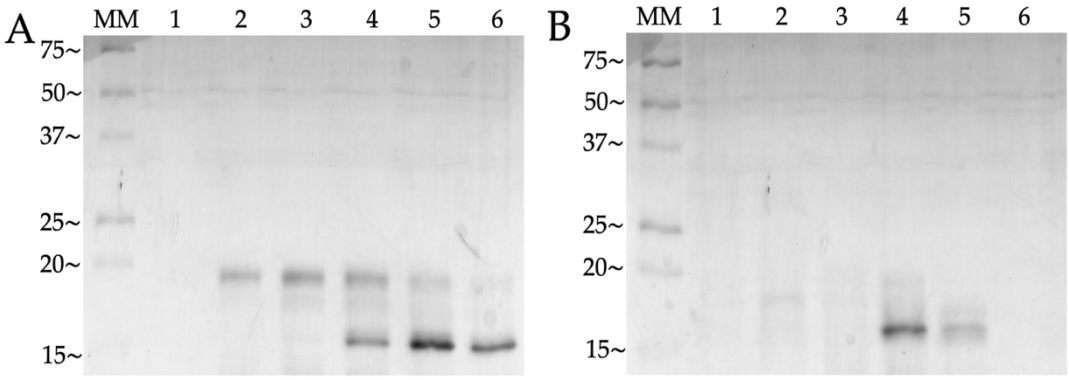


Figure S1. The fractions taken for the SDS-PAGE gel from ostrich (A) and emu (B) are listed in Table 1S with their respective volumes. The molecular weight marker (MM) corresponds to Prestained Protein Ladder (10 - 245 kDa).

Table S2. Fractions collected from injections made with 50 mM sodium citrate + 150 mM NaOH pH 4.0 at a flow rate of 0.5 mL/min. Fractions were collected every 1 mL and the volume in mL of the collected fractions is given. **Fxn:** Fraction.

Fxn	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C. *Acutus*	13	13.5	14	14.5	15	15.5	16	16.5
C. *Moreletti*	13	13.5	14	14.5	15	15.5	16	--

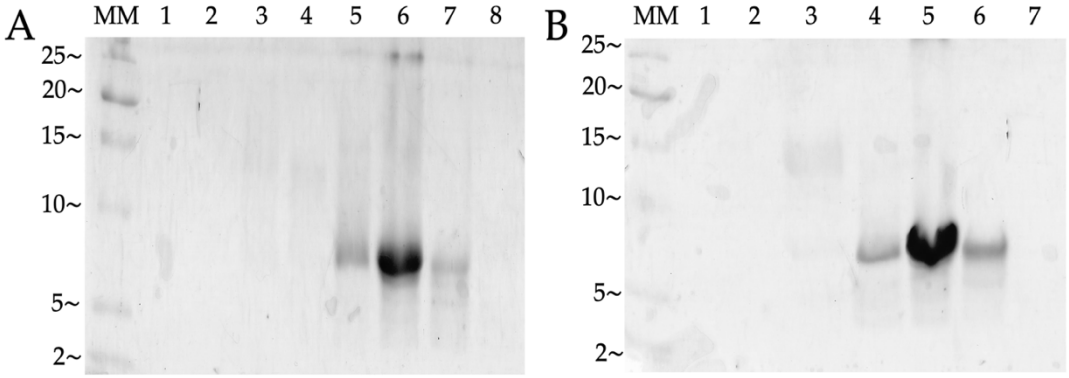


Figure S2. The fractions taken for the SDS-PAGE gel from crocodile *acutus* (A) and crocodile *moreletti* (B) are listed in Table 2S with their respective volumes. The molecular weight marker (MM) corresponds to Prestained Protein Ladder (10 - 245 kDa).

2. Mass Spectrometry of Intramineral Proteins

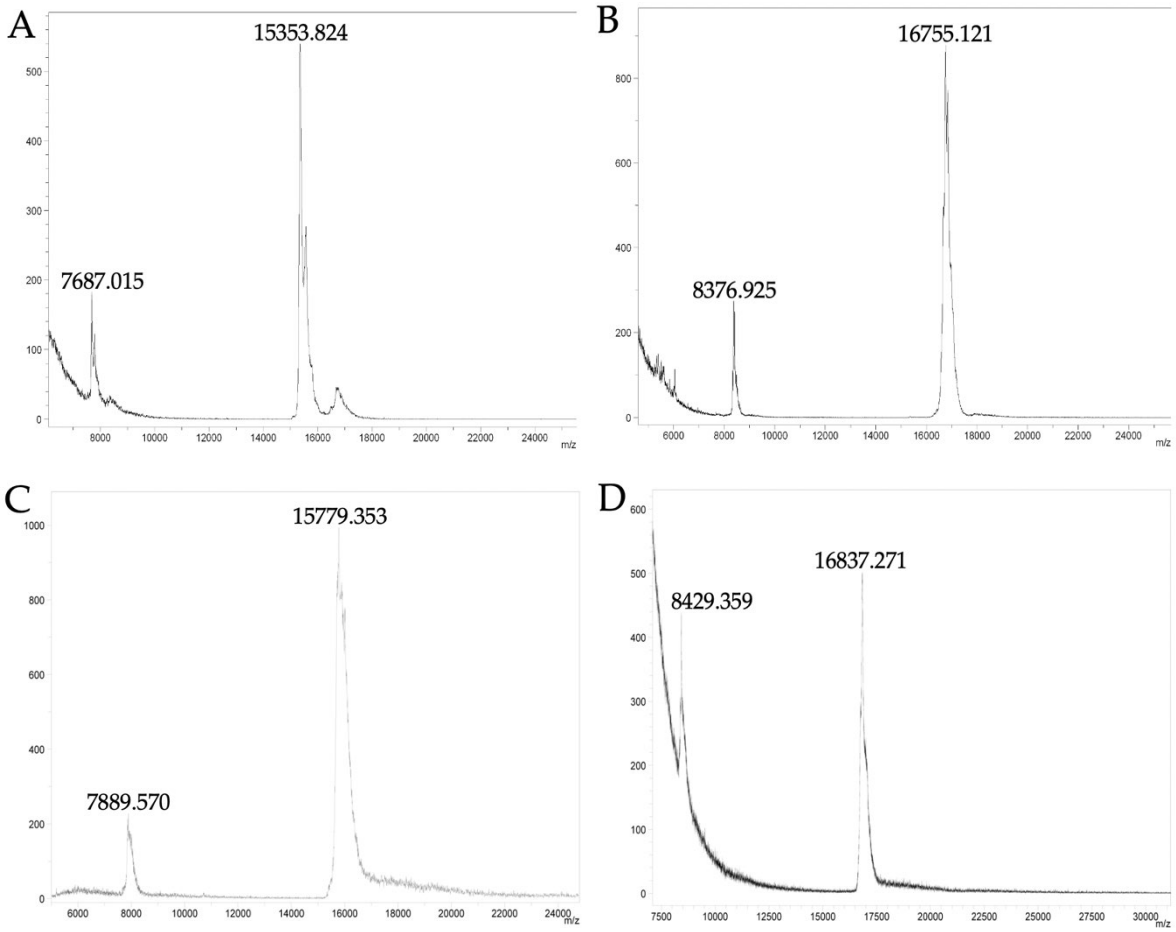
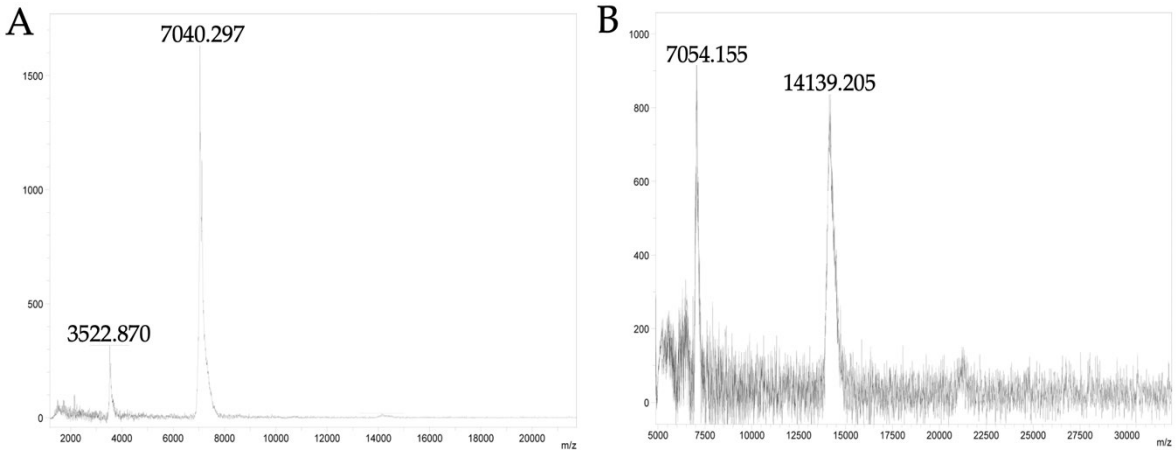


Figure S3. Mass spectra of intramineral proteins SCA-1 (A), SCA-2 (B), DCA-1 (C), and DCA-2 (D).



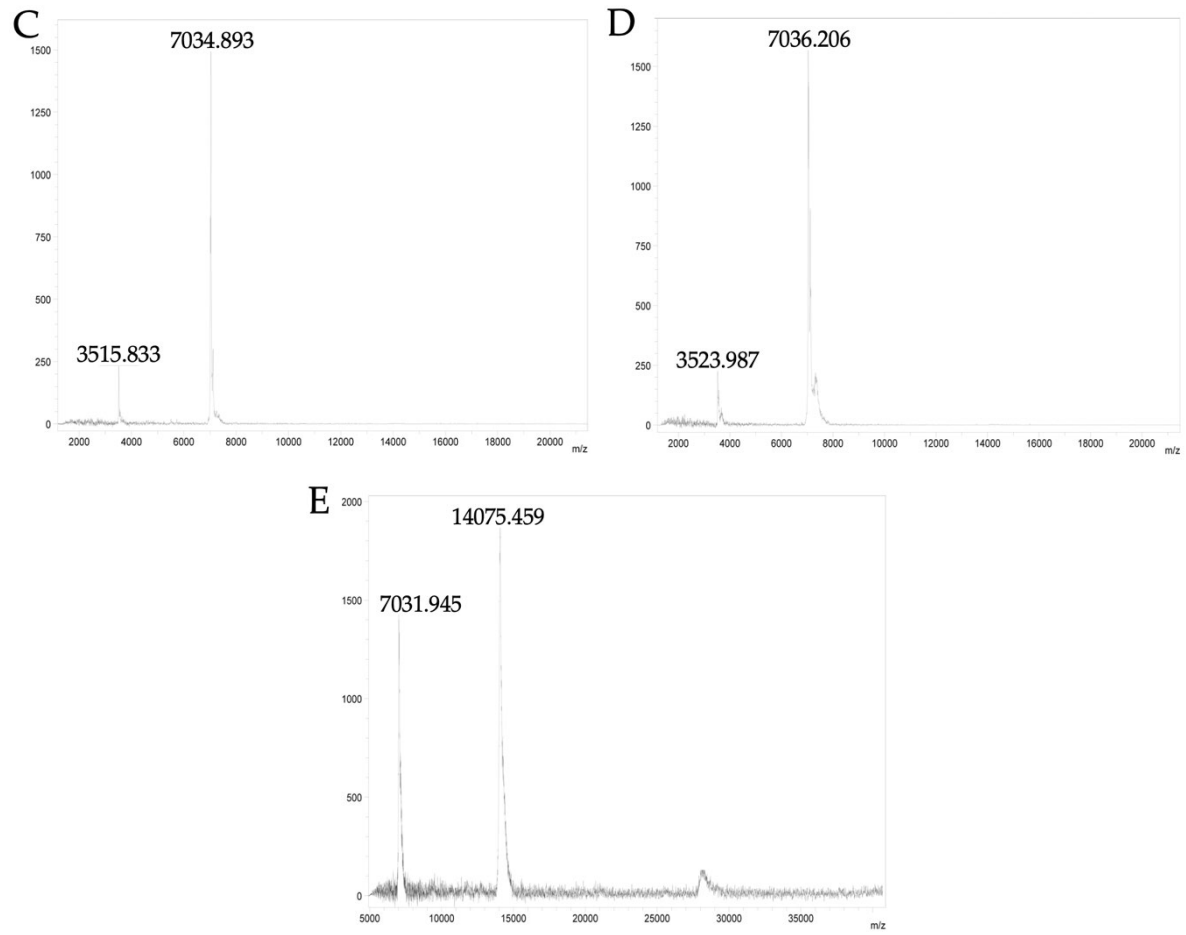
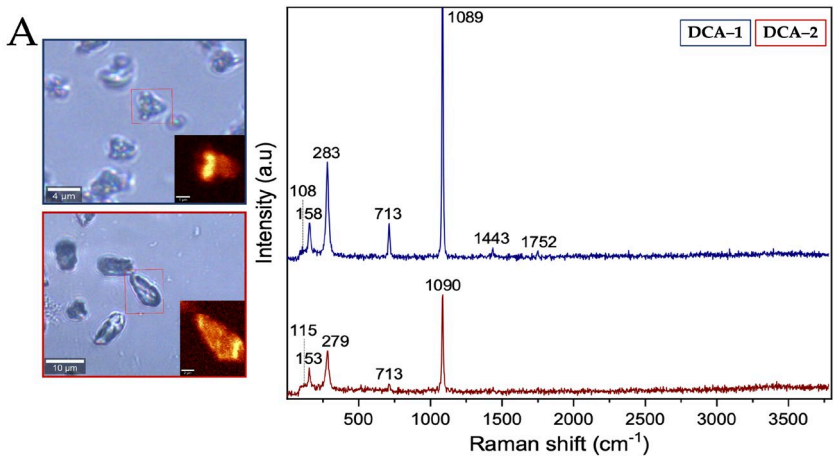


Figure S4. Mass spectra of intramineral proteins CCA-7 (A), CCA-14 (B), CCM-1 (C), CCM-2 (D), and CCM-3 (E).

3. Biomorphs Formation



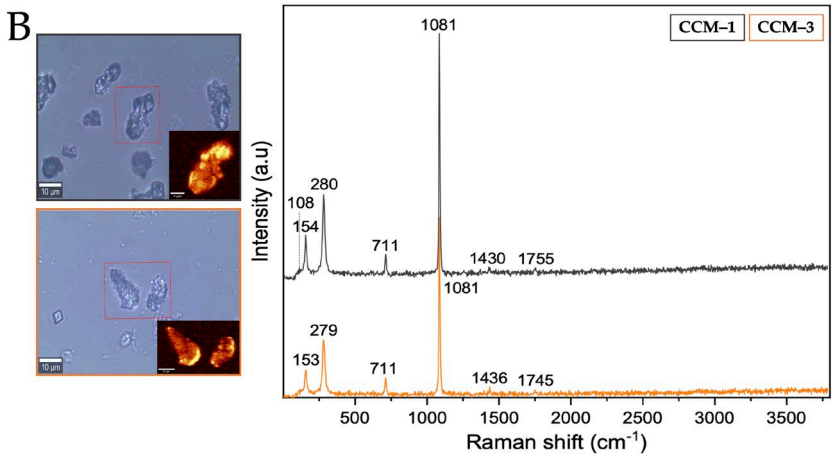


Figure S5. Raman spectra of calcium silica-carbonates synthesized. A: Dromaiocalcins; B: Crococalcins from crocodrile *moreletti*. The blue images are optical images, and the smaller ones correspond to the mapping performed on the biomorphs.

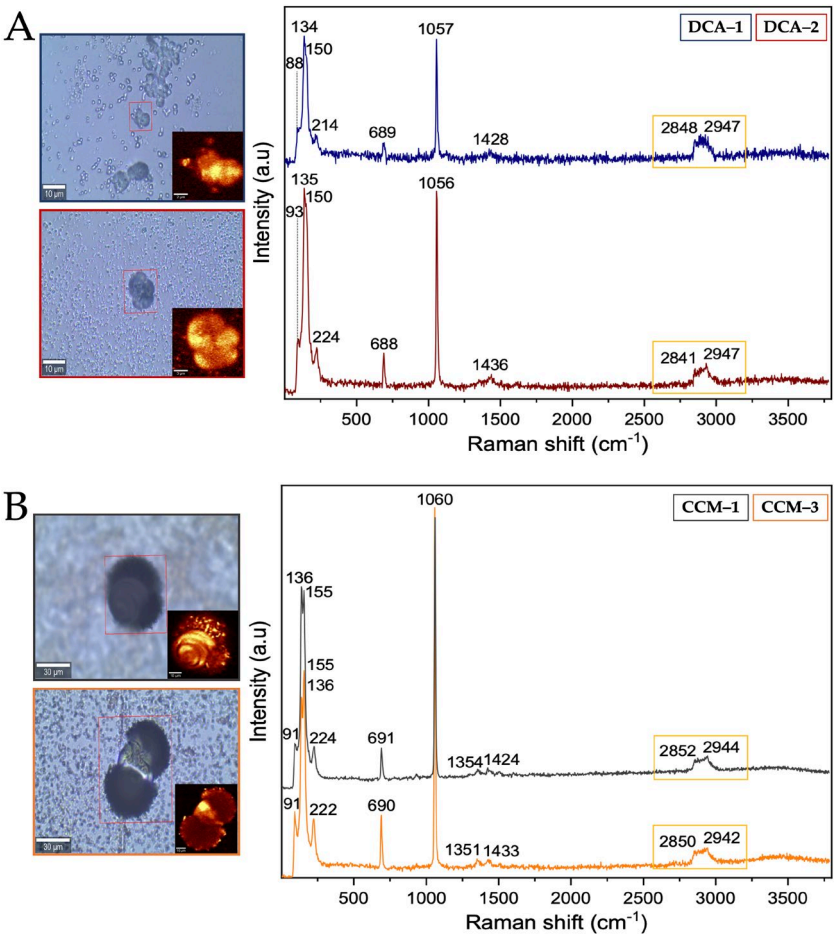


Figure S6. Raman spectra of barium silica-carbonates synthesized. A: Dromaiocalcins; B: Crococalcins from crocodrile *moreletti*. The blue images are optical images, and the smaller ones correspond to the mapping performed on the biomorphs. The yellow box indicates the protein signal.

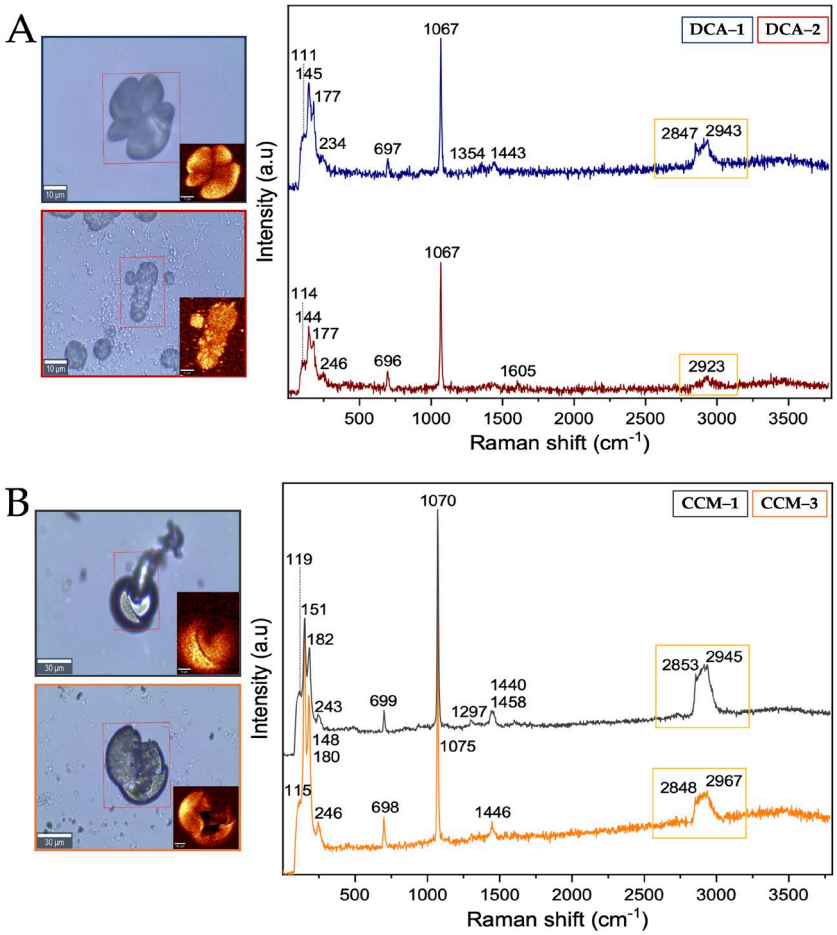


Figure S7. Raman spectra of strontium silica-carbonates synthesized. A: Dromaiocalcins; B: Crococalcins from crocodrile *moreletti*. The blue images are optical images, and the smaller ones correspond to the mapping performed on the biomorphs. The yellow box indicates the protein signal.

4. Elemental Analysis of the membranes of the ratite birds and reptiles eggshell

Table S3. Elemental percentage present in the membranes of the ratite birds and crocodiles eggshell.

Element	Ostrich	Emu	C. *Acutus*	C. *Moreletti*
C	55.97	55.86	55.01	59.50
N	11.56	15.47	17.18	11.80
O	25.30	24.26	23.86	24.39
S	4.03	3.70	3.95	4.31
Na	1.16	0.33	--	--
Mg	0.43	--	--	--
Cl	1.55	--	--	--
Si	--	0.38	--	--

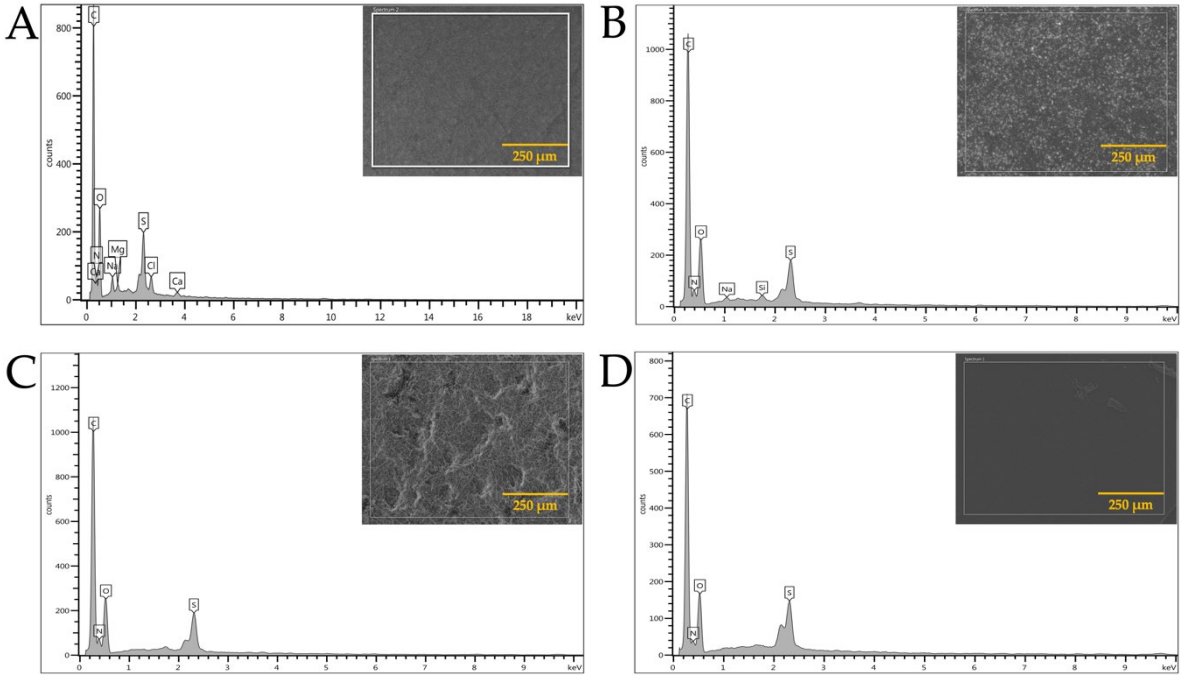
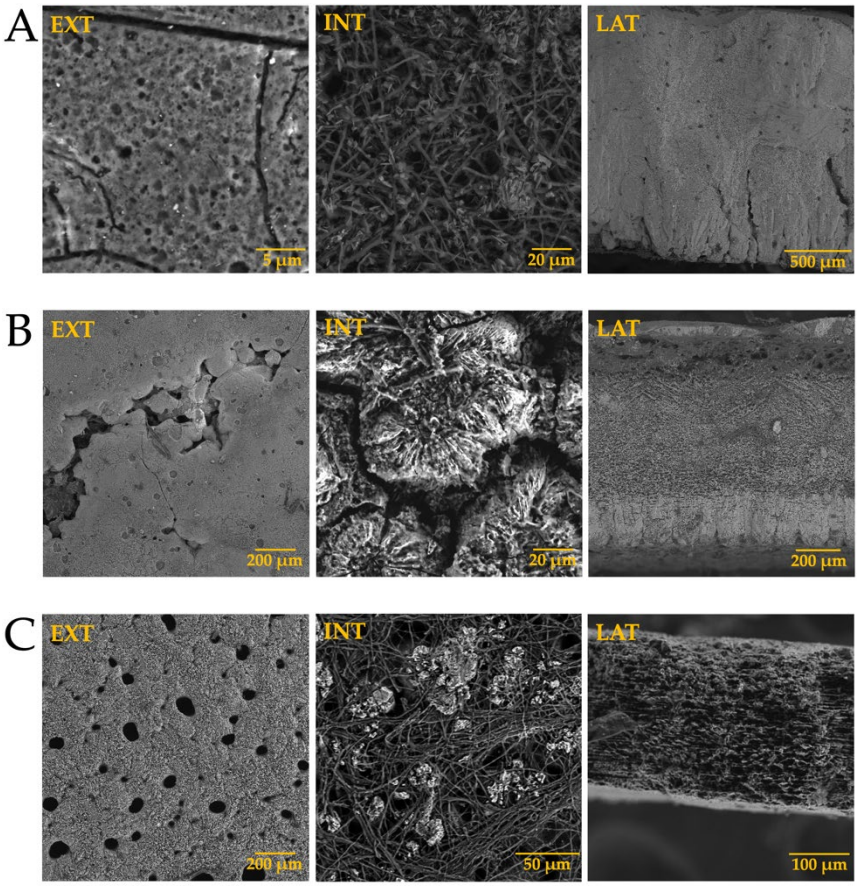


Figure S8. SEM-EDS images of the area outlined in white corresponding to the analysis performed on the eggshells membranes of ratite birds and crocodriles. A: Ostrich; B: Emu; C: C. *acutus*; D: C. *moreletti*.

5. Morphology of Eggshells of Birds and Crocodiles



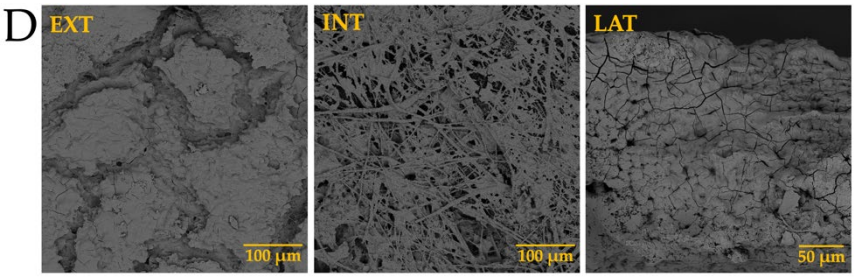


Figure S9. SEM images of ostrich (A), emu (B), crocodile *acutus* (C), and crocodile *moreletti* (D) eggshells. Ext: Outer part; Int: Inner part; Lat: Lateral/Intermediate section.

6. FTIR Analysis of Membranes of Birds and Crocodiles Eggshells

Table S4. Assignment of the FTIR vibrations of untreated membranes of ostrich before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; SCA: Struthicalcin.*

Group	WO	WT	SCA-1	SCA-2
Amide A	3290	3284	3278	3278
Amide B	3068	3068	3068	3068
νCH_2 ASYM	2960	2960	2954	2960
νCH_2 SYM	2929	2929	2935	2929
Amide I	1635	1638	1635	1635
Amide II	1528	1509	1516	1516
δCH_2	1446	1446	1442	1442
$\nu_3(CO_3)^{2-}$	1398	1409	1405	1398
Amide III	1238	1238	1235	1235
Blue Signal	1072	1098	1079	1086
$\nu_2(CO_3)^{2-}$	--	872	872	872

* The shift is given in cm^{-1}.

Table S5. Assignment of the FTIR vibrations of untreated membranes of emu before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; DCA: Dromaiocalcin.*

Group	WO	WT	DCA-1	DCA-2
Amide A	3278	3278	3274	3274
Amide B	3060	3060	3060	3060
νCH_2 ASYM	2960	2956	2960	2952
νCH_2 SYM	2926	2930	2926	2930
Amide I	1635	1632	1632	1632
Amide II	1528	1522	1522	1525
δCH_2	1448	1448	1448	1448
$\nu_3(CO_3)^{2-}$	1404	1398	1396	1396
Amide III	1234	1234	1234	1231
Blue Signal	1080	1080	1077	1077
$\nu_2(CO_3)^{2-}$	--	874	871	--

* The shift is given in cm^{-1}.

Table S6. Assignment of the FTIR vibrations of untreated membranes of crocodile *acutus* before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; CCA: Crococalcin.*

Group	WO	WT	CCA-7	CCA-14
Amide A	3322	3271	3315	3278
Amide B	3081	--	--	3081
νCH_2 ASYM	2966	--	--	2954
νCH_2 SYM	2929	--	--	2929
Amide I	1635	1635	1635	1635
Amide II	1540	1521	1521	1514
δCH_2	1445	1432	1432	1438
$\nu_3(CO_3)^{2-}$	1400	1407	1394	1400
Amide III	1241	1216	1229	1229
Blue Signal	1071	1077	1077	1077
$\nu_2(CO_3)^{2-}$	--	874	874	874

* The shift is given in cm^{-1}.

Table S7. Assignment of the FTIR vibrations of untreated membranes of crocodile *moreletti* before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; CCM: Crococalcin.*

Group	WO	WT	CCM-1	CCM-3
Amide A	3284	3278	3290	3278
Amide B	3081	3062	--	3068
νCH_2 ASYM	2960	2960	--	2960
νCH_2 SYM	2929	2922	--	2929
Amide I	1632	1635	1639	1632
Amide II	1517	1513	1517	1517
δCH_2	1444	1437	1434	1437
$\nu_3(CO_3)^{2-}$	1392	1399	1402	1409
Amide III	1236	1222	1218	1225
Blue Signal	1072	1072	1072	1076
$\nu_2(CO_3)^{2-}$	--	871	871	874

* The shift is given in cm^{-1}.

Table S8. Assignment of the FTIR vibrations of biocalcified membranes of ostrich before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; SCA: Struthicalcin.*

Group	WO	WT	SCA-1	SCA-2
$(OH)^-$	3367	--	--	--
Amide A	3265	3284	3284	3284
νCH_2 ASYM	2922	2966	2954	2960
νCH_2 SYM	2852	2929	2922	2922
Amide I	1635	1641	1648	1641
Amide II	1540	1527	1521	1534

δCH₂	1457	1438	1451	1445
ν₃(CO₃)²⁻	1432	1407	1407	1407
Amide III	1248	1241	1241	1235
ν₁,₃(PO₄)³⁻	1102 1026	1089 1019	1089 1026	1083 1019
ν₂(CO₃)²⁻	--	867	874	867
ν₂,₄(PO₄)³⁻	664 550	601 557	601 557	601 557

* The shift is given in cm^{-1}.

Table S9. Assignment of the FTIR vibrations of biocalcified membranes of emu before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; DCA: Dromaiocalcin.*

Group	WO	WT	DCA-1	DCA-2
(OH)⁻	3341	--	--	--
Amide A	3284	3271	3278	3278
νCH₂ ASYM	2992	2966	2960	2960
νCH₂ SYM	2929	2929	2922	2929
Amide I	1635	1635	1635	1635
Amide II	1534	1527	1527	1521
δCH₂	1445	1438	1445	1438
ν₃(CO₃)²⁻	1413	1407	1407	1407
Amide III	1235	1235	1235	1235
ν₁,₃(PO₄)³⁻	1096 1026	1096 1032	1089 1032	1089 1026
ν₂(CO₃)²⁻	--	867	867	874
ν₂,₄(PO₄)³⁻	601 557	601 557	601 557	601 557

* The shift is given in cm^{-1}.

Table S10. Assignment of the FTIR vibrations of biocalcified membranes of crocodile *acutus* before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; CCA: Crococalcin.*

Group	WO	WT	CCA-7	CCA-14
(OH)⁻	3360	--	--	--
Amide A	3290	3278	3284	3296
Amide I	1641	1635	1648	1648
Amide II	1534	1514	1514	1521
δCH₂	1470	1445	1445	1451
ν₃(CO₃)²⁻	1420	1407	1407	1407
Amide III	1235	1235	--	--
ν₁,₃(PO₄)³⁻	1083 1019	1077 1026	1089 1019	1089 1019
ν₂(CO₃)²⁻	--	874	867	867
ν₂,₄(PO₄)³⁻	595	601	601	601

	557	550	557	557

* The shift is given in cm^{-1}.

Table S11. Assignment of the FTIR vibrations of biocalcified membranes of crocodile *moreletti* before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; CCM: Crococalcin.*

Group	WO	WT	CCM-1	CCM-3
$(OH)^-$	3380	3385	3385	--
Amide A	3271	3278	3284	3278
νCH_2 ASYM	2966	2966	2966	2966
νCH_2 SYM	2935	2929	2935	2935
Amide I	1635	1635	1641	1635
Amide II	1534	1527	--	1514
δCH_2	1451	1451	1445	1445
$\nu_3(CO_3)^{2-}$	1413	1400	1407	1400
Amide III	1229	1235	1241	1235
$\nu_{1,3}(PO_4)^{3-}$	1071 1032	1077 1032	1077 1045	1077 1045
$\nu_2(CO_3)^{2-}$	--	--	874	874
$\nu_4(PO_4)^{3-}$				

* The shift is given in cm^{-1}.

Table S12. Assignment of the FTIR vibrations of biosilicified membranes of ostrich before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; SCA: Struthiocalcin.*

Group	WO	WT	SCA-1	SCA-2
$\nu(OH)^-$	3373	--	--	--
$\delta(OH)^-$	1622	1635	1635	1641
Amide III	1172	1172	1179	1179
$\nu(Si_2O)$ ASYM	1071	1058	1052	1052
$\nu(Si_2O)$ SYM	956	944	950	956
$\delta(Si_2O)$	--	798	798	791

* The shift is given in cm^{-1}.

Table S13. Assignment of the FTIR vibrations of biosilicified membranes of emu before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; SCA: Dromaiocalcin.*

Group	WO	WT	DCA-1	DCA-2
$\nu(OH)^-$	3385	--	--	--
$\delta(OH)^-$	1635	--	--	--
Amide III	1185	1172	1166	1172
$\nu(Si_2O)$ ASYM	1045	1052	1058	1058
$\nu(Si_2O)$ SYM	950	956	956	956
$\delta(Si_2O)$	791	798	791	791

* The shift is given in cm^{-1}.

Table S14. Assignment of the FTIR vibrations of biosilicified membranes of crocodile *acutus* before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; CCA: Crococalcin.*

Group	WO	WT	CCA-7	CCA-14
ν(OH)$^-$	3385	--	--	--
δ(OH)$^-$	1635	1635	1635	1635
Amide III	1172	1166	1159	1159
ν(Si_2O) ASYM	1058	1039	1045	1039
ν(Si_2O) SYM	944	944	956	950
δ(Si_2O)	791	791	791	791

* The shift is given in cm^{-1}.

Table S15. Assignment of the FTIR vibrations of biosilicified membranes of crocodile *moreletti* before and after synthesis of calcium biomorphs. WO: Without $CaCO_3$; WT: With $CaCO_3$; CCA: Crococalcin.*

Group	WO	WT	CCM-1	CCM-3
ν(OH)$^-$	3373	--	--	--
δ(OH)$^-$	1628	1635	1635	1635
Amide III	1172	1166	1191	1185
ν(Si_2O) ASYM	1045	1052	1052	1045
ν(Si_2O) SYM	944	963	963	963
δ(Si_2O)	791	791	791	791

* The shift is given in cm^{-1}.