

## Faraday Discussions

### Electronic Supplementary Information (ESI)

#### Scattering of ultraviolet light by avian eggshells

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#### Electronic supplementary information

**Table S1** – Table of samples: origin and identification of each egg sample investigated in this study.

Denomination in this study	Commercial branding	Species	Brand and retail shop
Beige hen eggs	-	Domestic hen ( <i>Gallus gallus domesticus</i> )	“Essential” from Waitrose (UK)
Brown hen eggs	“Burford Brown”	Domestic hen ( <i>Gallus gallus domesticus</i> )	“Clarence Court” from Waitrose (UK)
White hen eggs	-	Domestic hen ( <i>Gallus gallus domesticus</i> )	“Carrefour” (BE)
Turquoise hen eggs	“Old Costwold Legbar”	Domestic hen ( <i>Gallus gallus domesticus</i> )	“Clarence Court” from Waitrose (UK)
Duck eggs	“Gladdys-May’s Braddock Whites”	Duck	“Clarence Court” from Waitrose (UK)
Quail eggs	-	Quail	“Clarence Court” from Waitrose (UK)

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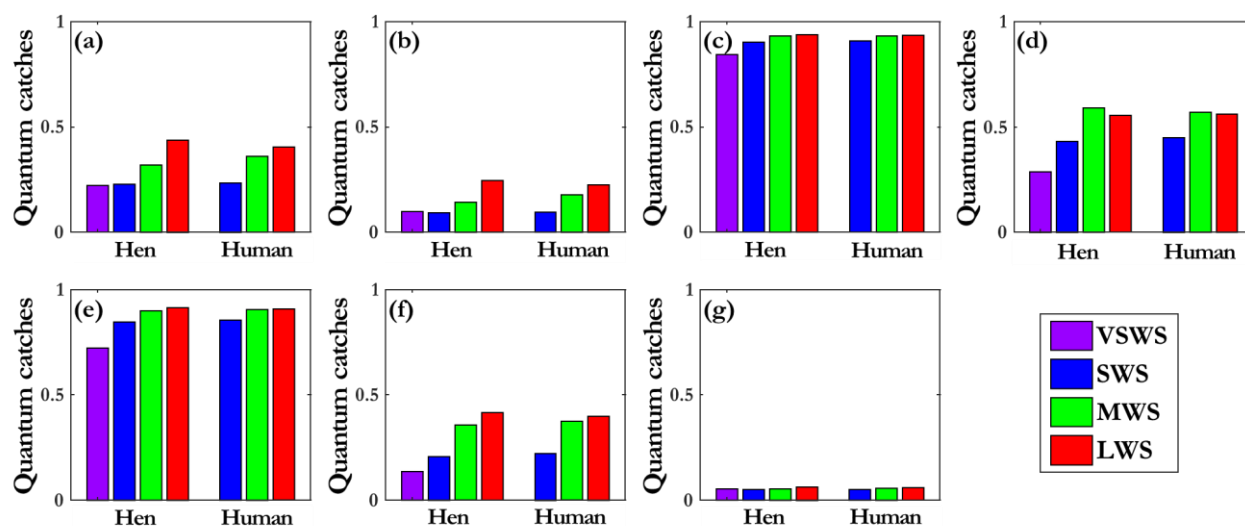
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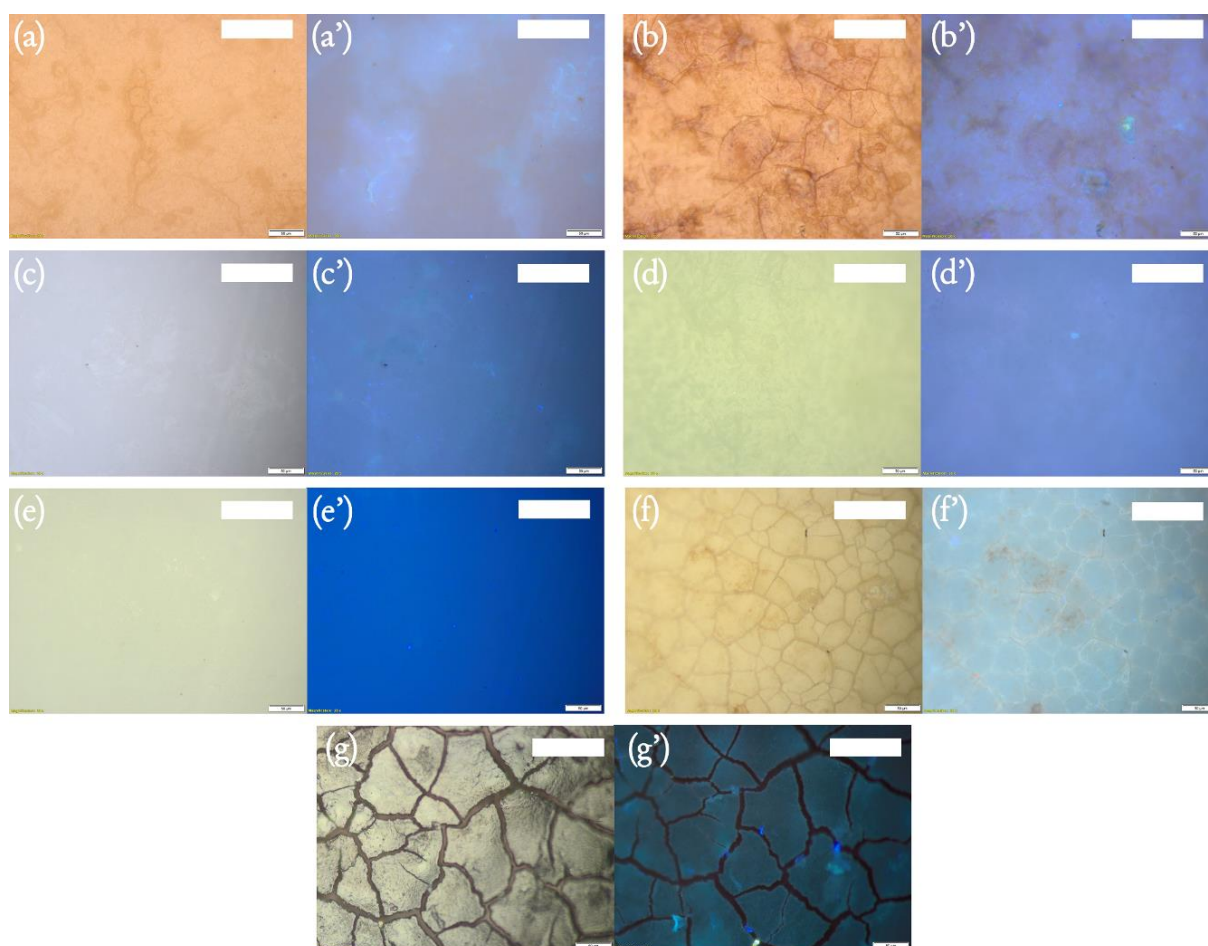
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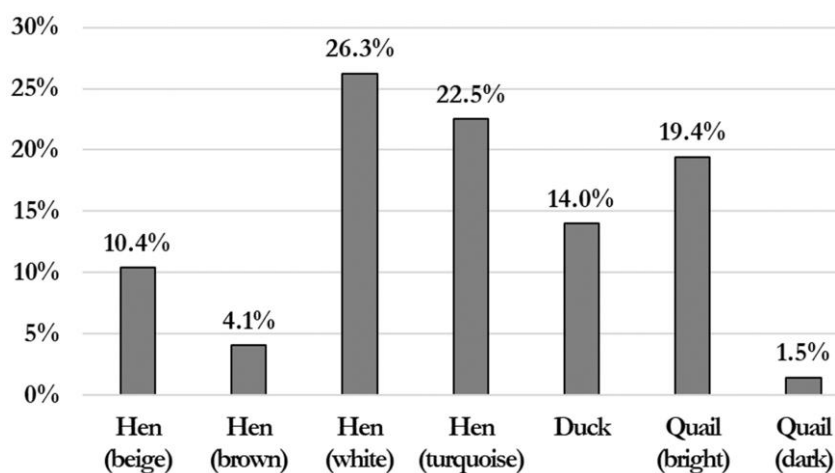
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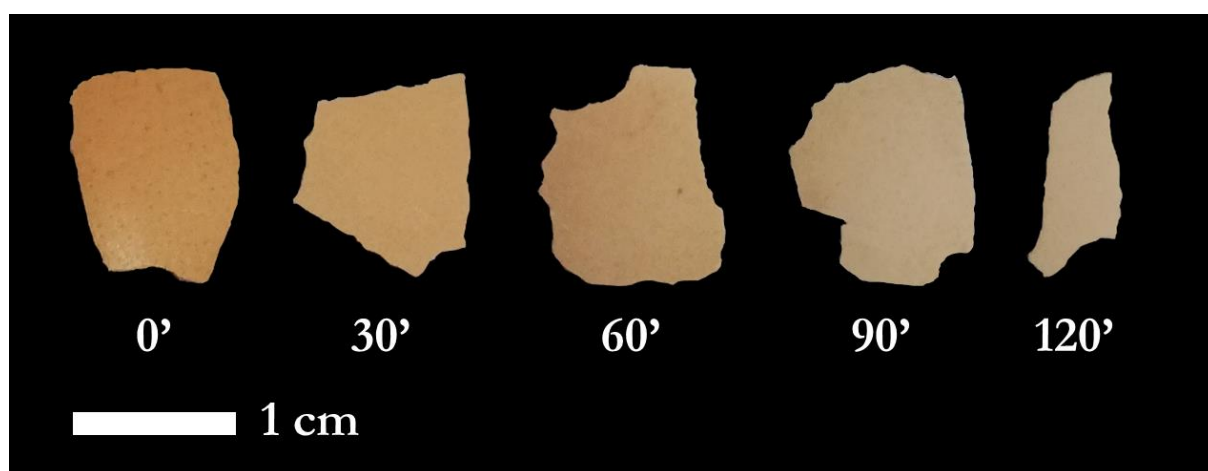
**Figure S1** – The visual appearances, which are very intense for humans are also very intense for hens, as shown here in terms of the quantum catches ( $\pm 0.05$ ) for hen and human being photoreceptor cells in the cases of beige (a), brown (b), white (c), turquoise (d) hen eggshells, duck eggshell (e) as well as the bright (f) and dark (g) areas of quail eggshells. Due to the high spectral intensity of the VSWS photoreceptor<sup>44</sup> and partly its overlapping with the 290-340-nm range, eggshells that are achromatic for humans such as (c) and (e) would be detected as chromatic by hens.



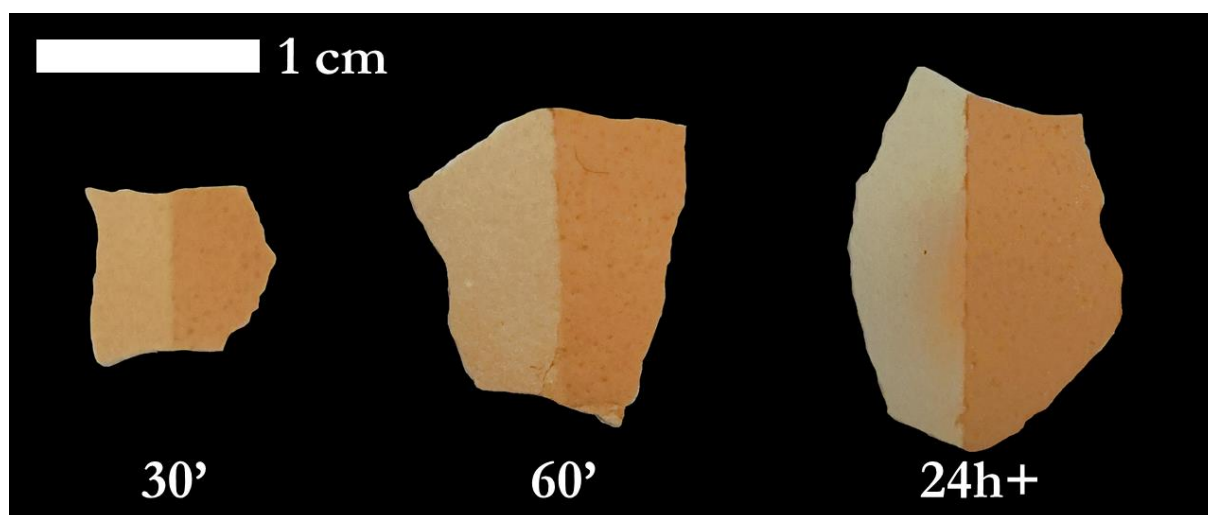
**Figure S2** – Optical and fluorescence microscopy observations of the surfaces of beige (a), brown (b), white (c), turquoise (d) hen eggshells, duck eggshell (e), bright (f) and dark (g) of quail eggshell. Each sample was observed under incident visible light (labels without apostrophe) and fluorescence configuration (labels with apostrophe). Scale bars: 100  $\mu\text{m}$



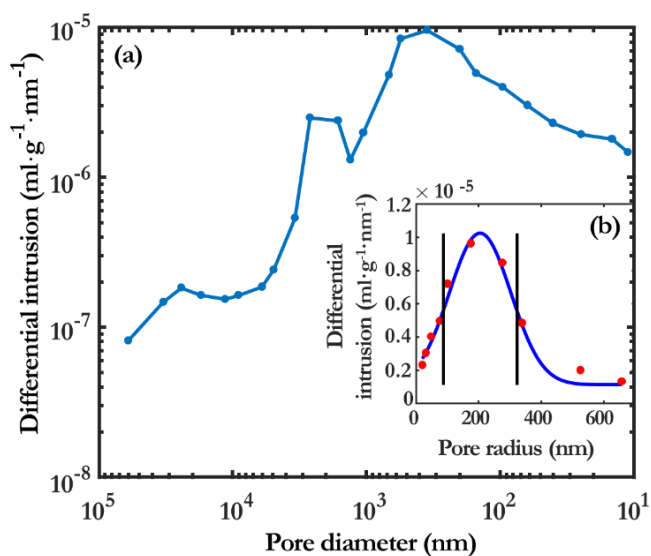
**Figure S3** – Fluorescence quantum efficiencies  $\phi_f$  ( $\pm 0.15\%$ ) were found to be rather low in all cases. Darker eggshells, namely, the beige and brown hen eggshells as well as the dark area of quail eggshells exhibit the lowest values, most likely due to stronger light absorption in the eggshell materials.



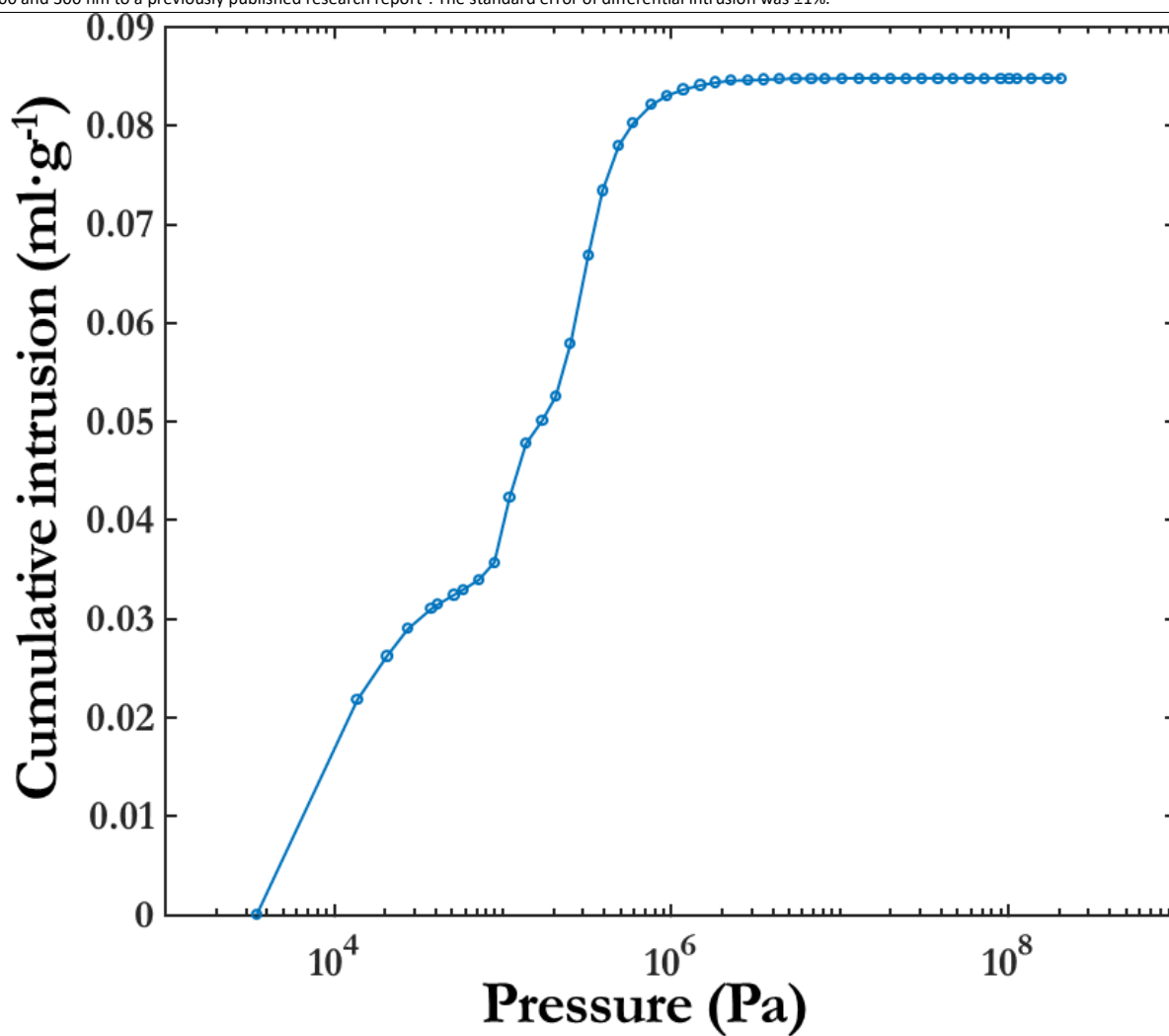
**Figure S4** – Pictures of beige hen eggshell samples treated with ethylenediaminetetraacetic acid (EDTA) 0.34 M for various durations. This process slowly thins down the cuticle that is known to contain pigments<sup>1</sup>. We observed a progressive loss in pigmentation with longer treatment durations.



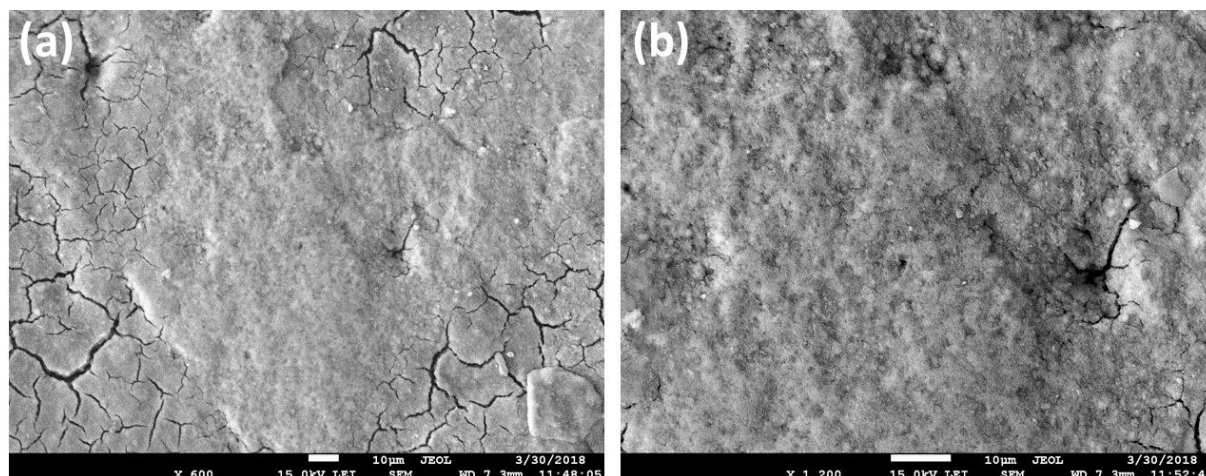
**Figure S5** – Beige hen eggshell samples used in order to assess the thickness of the dissolved layer with EDTA treatments for various durations. Tape was placed on the surface of the fragments prior to any chemically treatment. At the end of each treatment duration, the tape was removed and the cross-sections of the samples were observed by SEM, allowing to measure the difference in thickness between the protected and unprotected areas.



**Figure S6** – Mercury intrusion porosimetry allowed us to assess the pore diameter distribution (a). A Gaussian function was used to fit the measured distribution (b) and quite similar between 200 and 300 nm to a previously published research report<sup>3</sup>. The standard error of differential intrusion was  $\pm 1\%$ .



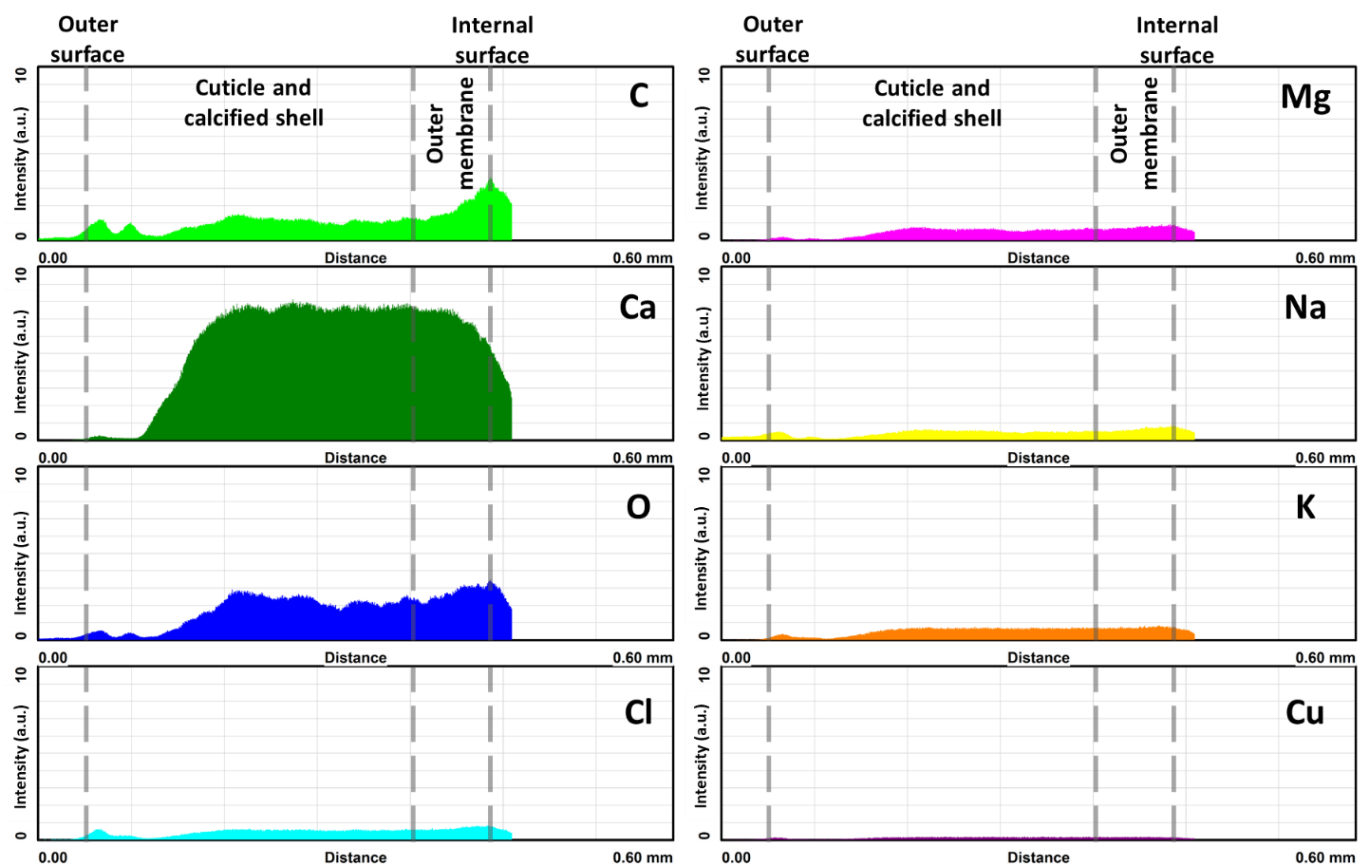
**Figure S7** – Porosimetry: mercury intrusion in beige hen eggshell samples as a function of the applied external pressure.



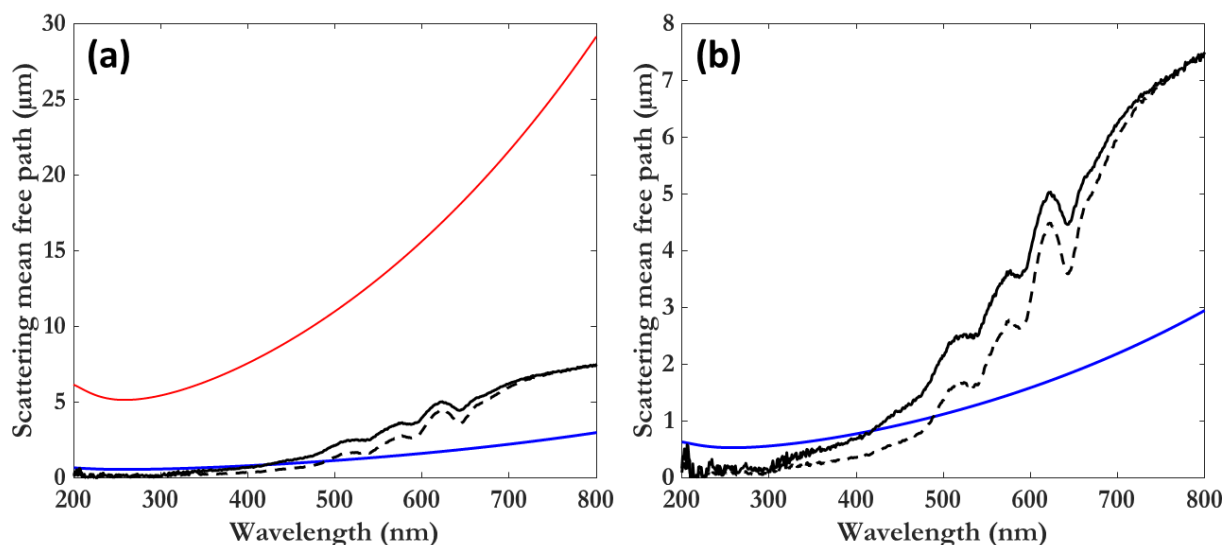
**Figure S8** – SEM observations of the surface of a beige hen eggshell sample with x600 (a) and x1200 (b) magnifications. The surface is quite rough. The pores observed in the calcified shell cannot be observed from the surface.

**Table S2** – Mass and atomic concentrations measured by EDX analysis of the cross-section of a beige hen eggshell. Measurements were performed for the whole cross-section and an arbitrary selected area of the calcified shell. The results indicate that the calcified shell is mostly composed of pure calcite. The error on mass concentration corresponds to the accuracy of the quantification of fitting coefficients of the EDX spectrum.

Element	Mass concentration on the whole shell cross section	Mass concentration on the calcified shell	Atomic concentration on the whole shell cross section	Atomic concentration on the calcified shell	Error on mass concentration
O	44.51%	46.73%	53.81%	58.90%	0.28%
Ca	36.52%	40.42%	17.62%	20.34%	0.13%
C	17.05%	12.03%	27.45%	20.20%	0.05%
Cu	0.43%	0.04%	0.13%	0.01%	0.91%
K	0.41%	0.24%	0.20%	0.12%	0.10%
Na	0.39%	0.17%	0.33%	0.15%	0.13%
Cl	0.39%	0.08%	0.21%	0.05%	0.08%
Mg	0.30%	0.28%	0.24%	0.24%	0.09%



**Figure S9** – The EDX analysis of the cross-section of a beige hen eggshell confirmed the presence of C, Ca and O in the chemical composition. In addition, other elements were found as traces.



**Figure S10** – Comparison between the scattering mean free path  $l_t(\lambda)$  calculated from Mie scattering cross-section of a single scatterer with a radius  $a = 145$  nm in the framework of an effective approach and the scattering mean free path assessed from the measured total transmittance. The scatterers density was assumed to be the one measured from SEM observations (a, red curve) and from porosimetry measurements (a&b, blue curve). The scattering mean free path was calculated from the total transmittance spectra (black solid line) measured from the beige hen eggshell samples that were chemically treated with EDTA 0.34 M for 2 hours and from these spectra with a Beer-Lambert correction (black dashed line). We observe satisfactory match between the scattering mean free path calculated from Mie theory with the scatterer density measured by mercury intrusion porosimetry and the mean free path calculated from the corrected total transmittance spectra in the UV range (b). The mismatch between the mean free path calculated from the pore density assessed by SEM observations and the one calculated from the corrected total transmittance spectra (a) is due to the cross-section observations performed by SEM that tend to underevaluate pore densities.

## Reference

- 1 D. Wilby, M. B. Toomey, P. Olsson, R. Frederiksen, M. C. Cornwall, R. Oulton, A. Kelber, J. C. Corbo and N. W. Roberts, *Journal of The Royal Society Interface*, 2015, **12**, 20150591.
- 2 D. C. Fecheyr-Lippens, B. Igic, L. D'Alba, D. Hanley, A. Verdes, M. Holford, G. I. N. Waterhouse, T. Grim, M. E. Hauber and M. D. Shawkey, *Biology Open*, 2015, **4**, 753-759.
- 3 N. La Scala Jr, I. C. Boleli, L. T. Ribeiro, D. Freitas and M. Macari, *Brazilian Journal of Poultry Science*, 2000, **2**, 177-181.