Electronic supplementary material

Non-linear optical spectroscopy and two-photon excited fluorescence spectroscopy reveal the excited states of fluorophores embedded in a beetle's elytra

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Electronic supplementary material 1:

Morphological characterisation

The electron microscopy analyses of the morphology of the scales covering the elytra were performed with a FEI (Hillsboro, OR, USA) Nova Nanolab 200 Dual-Beam scanning electron microscope (SEM). Elytra were cut into 5x5 mm^2 size pieces and fixed on the sample mount with conducting tape. 20-nm thick platinum layer was sputter-coated on the samples. The focused-ion beam (FIB) of the dual-beam SEM was used to cut the samples sharply and properly.

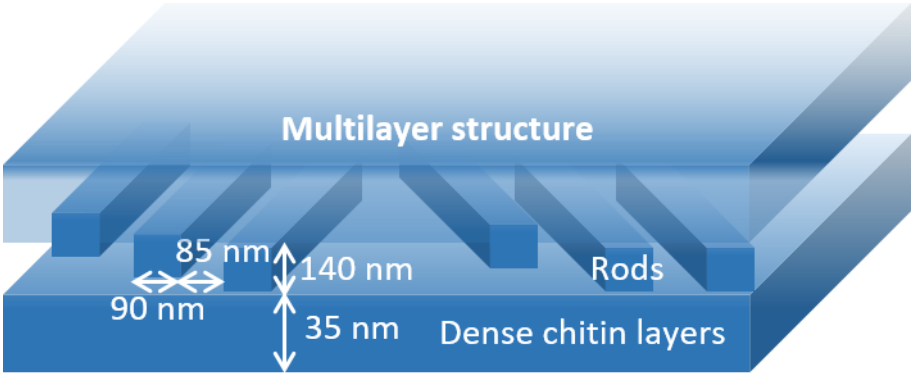

Figure S1: **Optical model of *H. coerulea*'s photonic structure.** The photonic structure comprised in the scales of *H. coerulea* is a multilayer containing 12 bilayer periods, each comprising a 35 nm-thick layer (assumed to be isotropic with a refractive index equal to 1.56) and a 140 nm-thick air-chitin layer. The air-chitin layers are made of the assembly of chitin rods of averaged width 90 nm, spaced by air gaps of 85 nm width. These structural dimensions were identified elsewhere [1,2].

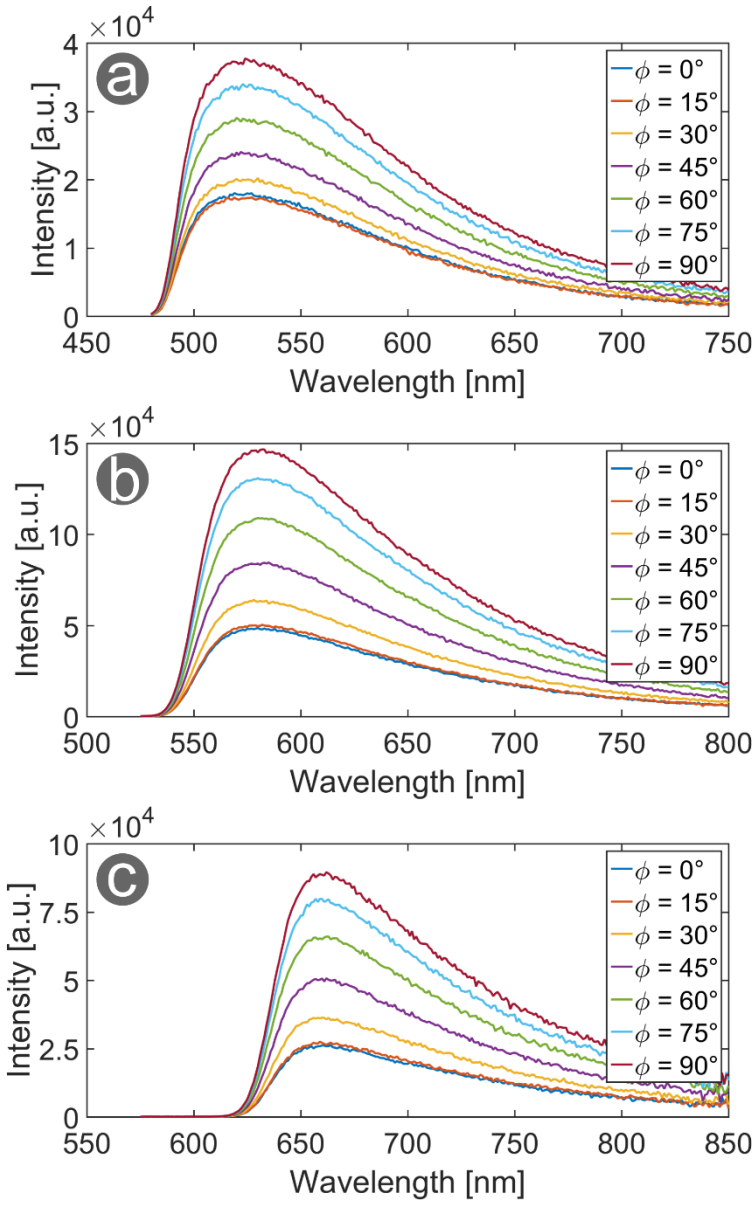

Figure S2: **OPEF response of *H. coerulea*'s elytra.** (a-c) The OPEF emission spectra from the fluorophores embedded within the beetle's photonic structure depend strongly on the linear polarisation states. ϕ is the angle of the rotating polariser. The excitation wavelengths are equal to (a) 450 nm, (b) 500 nm and (c) 550 nm, respectively.

λ_{em} (nm)	τ (ns) in dry state	τ (ns) in wet state
466	3.9 0.79	1.4
546	1.9	1.4

Table S1: **Decay times τ of the fluorophores embedded within the male *H. coerulea* beetle's photonic structures.** Measurements were performed in both dry and wet states for emission wavelengths λ_{em} located inside (466 nm) and outside (546 nm) the structure PhBG [2]. The incident 376-nm light beam and the emitted light formed 45° angles at either side of the direction normal to the sample surface.

Movie S1: **3D reconstruction of the TPEF signal from *H. coerulea*'s elytra.** The strong TPEF response from the elytra of the male *H. coerulea* with a 900-nm excitation light and a 15x magnification was used in order to perform a 3D reconstruction of the scales covering these elytra.

Supplementary References

1. M. Rassart, P. Simonis, A. Bay, O. Deparis, J.-P. Vigneron, “Scale coloration change following water absorption in the beetle *Hoplia coerulea* (Coleoptera),” Phys. Rev. E 80, 31910, 2009.
2. S. R. Mouchet, M. Lobet, B. Kolaric, A. M. Kaczmarek, R. Van Deun, P. Vukusic, O. Deparis and E. Van Hooijdonk, "Controlled fluorescence in a beetle's photonic structure and its sensitivity to environmentally induced changes," Proc. R. Soc. London B Biol. Sci. 283, 20162334, 2016.