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HAL Authorization

1 ***Viburnum tinus* Fruits Use Lipids to produce Metallic Blue Structural**

2 **Colour**

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16 **Summary**

17 *Viburnum tinus* is an evergreen shrub that is native to the Mediterranean region but cultivated
18 widely in Europe and around the world. It produces ripe metallic blue fruits throughout winter [1].
19 Despite its limited fleshy pulp,[2] its high lipid content[3] makes it a valuable resource to the small
20 birds[4] that act as its seed-dispersers[5]. Here, we find that the metallic blue appearance of the
21 fruits is produced by globular lipid inclusions arranged in a disordered multilayer structure. This
22 structure is embedded in the cell walls of the epicarp and underlaid with a dark layer of anthocyanin
23 pigments. The presence of such large, organised lipid aggregates in plant cell walls represents a new
24 mechanism for structural colouration and may serve as an honest signal of nutritional content.

25 **Results and Discussion**

26 The colours of fleshy fruits are thought to serve primarily to attract animal dispersers, and
27 understanding the origins and diversity of fleshy fruit colours provides insight into the ecological
28 interactions between plants and their dispersers.[7] *Viburnum tinus* (Adoxaceae) is a shrub native to
29 the Mediterranean region that is now cultivated around the world.[8] It produces large numbers of
30 metallic blue fruits throughout the year. Although a variety of bird dispersers consume *V. tinus*
31 fruits,[2] these serve as an especially important food source for birds during winter, including the
32 Eurasian blackcap (*Sylvia atricapilla*)[9] and the European robin (*Erithacus rubecola*).[10] While the
33 remarkable blue metallic appearance of *V. tinus* fruits (Figure 1) is commonly known and previously
34 reported,[11] the mechanism by which such blue colour is produced has not been elucidated. In a
35 review of earlier literature,[12] the colour of *V. tinus* has been attributed to the presence of
36 anthocyanin pigments.

37 The fruits of *V. tinus* (Figure 1c) reflect light directionally (producing its metallic appearance) in the
38 blue and UV spectral region (Figure S1). The polarisation of the reflected light is mostly retained
39 (Figure S2a-c&e), a property which indicates that the colouration is structural, rather than pigment-
40 based, originating in reflection from the highly structured cell wall of the outer epicarp (Figure 2a).
41 Dissection of this tissue releases a dark red anthocyanin pigment.[12] Light that is not reflected by
42 the photonic structure is absorbed by the dark red pigment underneath (Figure 2A, Figure 3C &
43 Figure S3E) This absorption prevents backscattering of light, increasing the prominence of the blue
44 reflection from the outer cell wall and therefore enhancing the visually blue appearance (Figure S3).

45 Thus the colour of *V. tinus* fruits results from a combination of a physical nanostructure that
46 selectively reflects blue wavelengths of light and a base layer of pigments that enhance the blue
47 colour.

48 A globular multilayer nanostructure composes the majority of the outer cell 49 wall

50 To characterize the nanostructures generating blue colour in *V. tinus* fruits, we used multiple
51 electron microscopy techniques. Scanning electron microscopy of fresh tissue (Figure 2A) clearly
52 shows the presence of a thick (10-30+ μm), multilayered structure, parallel to the surface of the fruit
53 and embedded in the cell wall of the outermost epicarpal cells. A waxy cuticle ($\sim 2\text{-}6\ \mu\text{m}$) on top of
54 the layered structure covers the fruit surface. The layered architecture occupies most of the outer
55 cell wall in the region between the cuticle and the cellulose-rich primary cell wall. The layers range in
56 thickness between 30-200nm (Figure 2) and extend across the whole cell. Transmission electron
57 microscopy (Figure 2) reveals that this architecture consists of many layers of small vesicles that
58 differ in electron scattering power and refractive index from the matrix. Imaging by SEM and TEM
59 (Figures 2, 3 & S4) indicates that the matrix appears to contain key components of typical plant cell
60 walls, namely cellulose and hemicelluloses. Staining with ruthenium red (Figures 3D & S4A) shows a
61 significant pectin content, and electron diffraction demonstrates the presence of cellulose by
62 characteristic diffraction rings of the native cellulose crystal (Figure S4). Although the refractive
63 index contrast layers are discrete and remain distinct from one another, considerable disorder is
64 introduced by non-parallel neighbouring layers and the irregularity of their globular structure. Serial
65 tomography (Figure 2E) of the epidermal cell wall reveals that these globular vesicles are organised
66 in merged layers through which the cellulosic cell wall matrix remains connected by bridges and
67 strings (Figure 2B; a 3D rotation model of this structure is available in the supplemental material
68 Video S1).

69 Evidence for the presence of lipids in the globular contrast phase

70 We demonstrated that the globular multilayer structure in the *V. tinus* fruit epidermis is composed
71 of lipids embedded in a cell wall matrix using a variety of methods. As solvency in nonpolar organic
72 solvents is a well-defined hallmark of lipids, we imaged unfixed cryo ultrathin sections of the fruit
73 epidermis before and after exposure to chloroform.[13] TEM images from the same sample region
74 before (Figure 3A) and after (Figure 3B) chloroform exposure show that the globular structure has
75 been removed by the treatment. In the latter image, the contrast of the globular multilayer phase is
76 reduced and the empty structures within the matrix remain visible. In comparison, exposure to
77 water did not alter the ultrastructure or image contrast of the globular multilayer, indicating that the
78 material is extractable only with non-polar solvents. Secondly, when imidazole buffered osmium
79 tetroxide, which binds to lipids, [14][15] was used during the chemical fixation process, the globular
80 layers were preferentially stained (Figure 2) confirming their lipidic nature. In contrast, when
81 ruthenium red was used (which binds to pectin[16]) the cell wall matrix was preferentially stained
82 while the globular structure was washed away in the absence of imidazole buffer (Figure 3D).
83 Additionally, a thin section of the epicarp was stained with Sudan Black B[17] which visibly stains
84 lipids observed in the thickened cell wall in Figure S2D.

85 For all of the staining methods, we observed dark outlines around the globules (Figure 3E, Figure
86 S4A). This could indicate the presence of a lipid membrane, which might be required at the interface
87 between hydrophobic molecules and the hydrophilic polysaccharides of the secondary cell wall.[18]

88 Lipids comprise a diversity of molecular structures, generally classified as waxes, fats and oils,
89 depending on their melting points.[19] Waxes are regularly found on the surface of the plant
90 epidermis, making up the water-resistant waxy cuticle. Although waxes also include a diversity of
91 molecular structures,[20] at least one prevalent component, alkanes,[21] are sufficiently indigestible
92 to be a useful diet indicator in faecal samples.[22] In contrast, oils and fats are a vital nutritional
93 resource as they contain much more energy per volume than is stored in starch or proteins.[15] Fats
94 are generally found in large oil bodies inside cells of storage organs like seeds. In the case of *V. tinus*
95 fruits, the close proximity of the globular structure to both the large energy-rich seed and the waxy
96 external cuticle[23] makes the distinction between waxes and fats a particularly important one for
97 understanding both the functional significance and developmental origins of the structure. In order
98 to further identify whether the lipid globules are indigestible waxes or nutritious fats and oils, we
99 used a variety of light-microscopy stains. Fixed sections were incubated with Nile Blue A,[24] which
100 stains the globule-rich region of the cell wall in *V. tinus* a blue or blue/purple-colour (Figure 3C). This
101 indicates that the globules are more likely to be free fatty acids than cutin polymer, which would
102 stain pink or red.[24]

103 Additionally, an electron diffraction diagram of the globular multilayer structure (Figure S4C-D)
104 shows a sharp ring pattern, differing from the diagram of a cellulose cell wall with the characteristic
105 two rings of cellulose crystals (Figure S4D). This pattern indicates that the lipid bodies are likely
106 crystalline and therefore are homogenous monomeric lipids rather than polymerised molecules such
107 as cutin, the waxy cuticle component, making them likely to be digestible. This raises the question of
108 whether the lipids are derived from the cuticularisation process, arrested before polymerisation,[25]
109 or derived from the internal nutritional lipids. Further extraction and characterisation would be
110 required to ascertain the chemical composition and the developmental and evolutionary origins of
111 these lipid bodies.

112 The biphasic organisation of the cell wall maintains hydrophilic channels spanning the globular lipid
113 structure. Given the high nutritionally relevant lipid content of the fruit,[3] and the very thin layer of
114 flesh that composes the mesocarp, the proportion of lipid stored in the epicarp could be substantial,
115 but this remains to be determined.

116

117 Models of the optical reflectance from lipid globular multilayer

118 In order to confirm that the observed mixed structure composed of a cellulosic matrix and layered
119 lipid globules is responsible for the blue reflectance of *V. tinus* fruits, we modelled its optical
120 response. To do this we investigated two mathematical models – (i) a 2D array of spheres, and (ii)
121 averaging over many 1D biphasic multilayers. Disorder was introduced into the models and
122 compared to experimental data.

123 An inverse design algorithm was used to model the structure in 2D as a series of globular
124 accumulations; in Figure 4A-C each of the schematics corresponds to the adjacent modelled
125 reflected spectrum.[26] This algorithm allows us to independently introduce different types of
126 disorder into the globular multilayer by tailoring the size and structure factor, i.e. the Fourier
127 transform of the positions of the particles. We studied the optical response of layered lipid globules
128 with different amounts of variation in globule diameter (Figure 4A), disorder in the angle between
129 adjacent globules (parameter S_p , Figure 4B, no disorder produces a flat plane), and disorder in the
130 average distance between adjacent globules (parameter S_k , Figure 4C). The introduction of different
131 types of disorder (Figure 4A-C) always produced the same effect on the optical response of the
132 globular multilayer, namely reducing the peak intensity.

133 Therefore, rather than dealing with each disorder element individually, the structure and material
134 composition of the *V. tinus* cell wall was approximated as disordered 1D multilayers with refractive
135 indices corresponding to cellulose ($n = 1.55$) [27] and a typical plant lipid ($n = 1.47$). [28] The model
136 includes water immersion conditions, and the existence of a dark anthocyanin absorptive pigment
137 underneath. The anthocyanin absorption spectrum used here is the cyanidin-3-glucoside extracted
138 from bilberry (*Vaccinium myrtillus*), [29] which is one of the primary anthocyanins previously
139 identified in *V. tinus* (the other being cyanidin 3-(200-xylosylglucoside)-5-glucoside). [12]

140 The statistical distribution of layer characteristics was measured from TEM cross-sections for
141 initialisation in the multilayer model. The distributions of thicknesses of both materials are shown in
142 Figure 4D. However, the effective model used a lower degree of disorder in layer thickness (s.d.
143 =25nm rather than measured s.d. = 45nm) which is attributed to a small sample size, TEM
144 artefacts [30] and long range order. The modelled reflectance using the averages over 1D multilayers
145 is shown in Figure 4E.

146 Introducing disorder, as observed in cross-section measurements, into the model of a coherent
147 ordered reflector broadens its reflectance band, in agreement with previous studies. [31] The
148 broadband, asymmetric peak and angle-independence improves the fit of the resulting model to the
149 real reflectance measured from *V. tinus* fruits, indicating that the model represents a good
150 approximation to the *V. tinus* measurements. Due to its simplicity, the model is not meant to
151 perfectly replicate the experimental measurements, but rather to indicate the validity of the
152 disordered multilayer producing the optical response measured in *V. tinus*. The disorder in the
153 multilayer in the form of boundaries of the globular multilayer that are non-perpendicular to the
154 optical axis is consistent with the visibility of diffuse, wide-angle blue scattering observed in cross-
155 section, as imaged in Figure S2E.

156 Implications of lipid-based structural colour for the honest signalling of 157 nutrition

158 The colouration of *V. tinus* fruits is very striking to the human visual system. Comparison with blue tit
159 spectral sensitivity (reproduced in Figure 1D [6]) shows that it falls well within the visually relevant
160 range for relevant birds. The visual background for the fruit is predominantly the green leaves of *V.*
161 *tinus*, the dominant pigment of which is chlorophyll. Chlorophyll has a broad spectral signature, with
162 a peak at 550nm, and negligible reflectance below 500nm, making the colour of *V. tinus* fruits
163 chromatically contrasted against foliage, as shown in Figure S3D. Birds are strongly responsive to
164 visual stimuli, [32] and a predominant function of fruit colouration is to attract animals that carry out
165 seed dispersal. [33][34] Thus, the lipid-based structural colour of *V. tinus* fruits could act as a visual
166 signal for foraging birds.

167 Since birds likely use fruit colour to help them determine which fruits to consume, the colours of
168 fruits may function as an honest signal of their nutritional reward. Honest signalling in fruits has
169 been described, but the results are inconsistent. In some species in the Atlantic forest of Brazil, dark
170 colouration corresponds with a carbohydrate-rich nutritional reward [35] whereas in other species in
171 the Mediterranean, dark colouration has been associated with a lipid-rich nutritional reward. [36]
172 The correlation between anthocyanin content and dark colour is thought to derive from shared
173 biochemical pathways between anthocyanin and sugar synthesis. [35] Greater anthocyanin content
174 typically makes fruits darker [37] although we know of no such biochemical explanation for the
175 correlation between dark colouration and lipid content.

176 We posit that blue fruit colour in *V. tinus* could serve as an honest signal of nutritional lipid content
177 owing to fact that in this system lipids both create the colour and provide nutrition The lipids in the
178 photonic structure are probably short-chain fatty acids, which are digestible by birds and thus
179 contribute in part to the overall nutritional content. We did not determine what proportion of the
180 high lipid nutritional reward in *V. tinus* fruits[3] is due to lipids in the photonic structure rather than
181 lipids occurring elsewhere in the mesocarp.

182 Honest signalling systems are often thought to be expensive due to the cost of producing the signal.
183 [36][38]. In this case, however, the interests of the signaller and the receiver are aligned: chromatic
184 contrast enhances visibility for birds and signals a more valuable nutritional content to the bird, both
185 features that are likely to increase dispersal. Although there is a cost associated with producing a
186 blue photonic structure (the energy invested in making the lipids), that cost also provides direct
187 benefits in the form of better advertising and better nutritional reward.

188
189 A disordered lipid multilayer as we report here in *V. tinus* has not been described previously in a
190 biomaterial. However, the nanostructure in *V. tinus* may help us understand colouration in two
191 other species with structurally coloured fruits (*Elaeocarpus angustifolius*[39] and *Delarbrea*
192 *michieana*[40]), which show strong morphological parallels. For instance, *D. michieana* is described
193 as having an iridosome, composed of “bubble-like” structures, that visually appears similar to the
194 architecture we observe in *V. tinus*. [40] Should these other species prove to use a similar structure
195 to that of *V. tinus*, it would provide an intriguing example of parallel evolution in distantly related
196 plant lineages.

197

198 Conclusions

199 The architecture (a disordered multilayer reflector, generated by a biphasic structure of lipids and
200 cellulose cell wall) in the epidermal cell walls of *V. tinus* fruits is unlike that of any previously
201 described structurally coloured fruits, or any other known structurally coloured material. The
202 coincidence of the striking colouration and high nutritional content of the coloured material itself
203 suggests that it may be an honest signal of nutritional reward evolved as a result of biotic selection.

204

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215

216 Author Contributions

217 Conceptualisation, R. M., M.S.A., E.M., B.J.G., S.V.; Investigation: Optical Characterisation, R.M.;
218 Electron Microscopy, M.S.A., Y.O., P.J.R., C.P., M.C. ; Numerical Modelling, R.M., G.J.; Composition
219 Analysis, M.S.A., Y.O.; Formal Analysis, R.M., M.S.A.; Resources, R.M., M.S.A., E.M.; Writing – Original

220 Draft, R.M.; Writing – Review & Editing, All Authors; Visualisation, R.M., M.S.A., Y.O., G.J.;
221 Supervision, B.J.G., M.D., S.V.;

223 Declaration of Interests

224 The authors declare no competing interests.

226 Figure Legends

227
228 **Figure 1. Optical appearance of ripe *V. tinus* fruits.** (A) A habitat photograph of ripe fruits on *V. tinus*
229 shrub in natural light displaying a metallic blue appearance. An additional macro-photograph and
230 UV-isolated image are shown in Figure S1. (B) Microscopic image of the fruit surface in reflected
231 light, showing the angle-dependence of colour intensity over the curvature of each cell. Additional
232 polarisation microscopy response and cross-sectional microscopy are shown in Figure S2. (C)
233 Macroscopic UV-Vis reflectance spectrum taken by a double-ended probe and calibrated to a silver
234 mirror reference. Additional macroscale spectra comparing fruit and leaf reflectance are included in
235 Figure S3D. (D) Predicted blue tit (*Parus caeruleus*) spectral sensitivity from cone response, oil
236 droplets and ocular media, reproduced from [6]. Sensitivities shown as a reference for the visual
237 relevance of the fruit signal to potential fruit dispersers. The x-axis is identical in both plots. The blue
238 tit visual system is chosen for phylogenetic relatedness with a principal *V. tinus* frugivore, *Sylvia*
239 *atricapilla* (Eurasian blackcap).

240 **Figure 2. Electron microscopy and reconstruction of internal 3D architecture** (A) Block-face SEM
241 showing epidermal cell with internal cavity filled with pigmented contents. Most of the cell wall
242 constitutes the globular multilayer. Labels: wc – waxy cuticle, gm – globular multilayer region, mc –
243 monophasic (cellulosic) cell wall, cc – cell content. The dark red pigment is present in the cell content
244 'cc', and is seen in a light microscope cross-section in Figure S3E. A reduced magnification showing
245 multiple cells is shown in Figure S4B. (B) TEM of the globular multilayer in the outer cell wall matrix;
246 the cell outer surface is above the top edge. (C) Higher magnification TEM of globules shown in (B).
247 (D) 3D reconstruction of globular layers reconstructed from TEM serial tomography using the
248 isosurface function in iMod. The globular multilayer forms a merged plate-like structure rather than
249 isolated globules. Full rotation video is shown in Video S1. (E) Reconstruction of globular inclusions
250 using three joined serial tomograms parallel to the curved fruit surface. The plate-like structure
251 parallel to the surface is visible, along with the bridges traversing it.

252 **Figure 3. Identification of cell wall multilayer components.** (A-B) A cryo-ultrathin section of the
253 same portion of the globular multilayer region imaged before (A) and after (B) extraction with
254 chloroform, showing the removal of the electron-dense internal lipid phase in the globular layers.
255 Arrows indicate the same location on the sample, which appears inverted due to the loss of stained
256 lipid. (C) Nile Blue A staining of the fruit epicarp observed with transmitted light microscopy showing
257 the cellulosic cell walls (dark blue), the outer cuticle (pink) and the mixed layer (purple) in the
258 multilayer. (D) TEM image after ruthenium red staining indicates that pectin is present in the cell
259 wall matrix, a higher magnification image is given in Figure S4(A). (E) TEM with lipid staining showing
260 dark outlines around the globules. ew, epicuticular wax, wc – waxy cuticle, gm – globular multilayer
261 region, mc – monophasic (cellulosic) cell wall, cc – cell content.

262 **Figure 4 Numerical models of multilayers and disorder.** (A-C), Simulated optical response of 2µm
263 thick, 2D models with different types of disorder as indicated in schematics (A1, B1, C1). (D) A
264 histogram presenting data showing the distribution of layer widths for both cellulose and lipid layers,
265 measured from analysis of TEM cross-section profiles. (E) Numerical model of the lipid-cellulosic

266 structure as a 1D disordered multilayer: 58nm (lipid layer), 67nm (cellulose layer), s.d. = 25nm. The
267 experimentally measured *V. tinus* spectrum is shown again for comparison.

268 **STAR Methods**

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270 **Resource Availability**

271 This study did not generate new unique reagents, nor any unique datasets or code.

272

273 **Experimental Model and Subject Details**

274 *V. tinus* fruits were imaged with optical and electron microscopes ripening over the course of five
275 years. We estimate fifteen separate occasions each for optical and electron microscopic analyses,
276 the majority of which treated several samples from different fruits and individual plants. Samples
277 were collected from private and college gardens around Cambridge and Bristol. TEM and SEM
278 measurements were consistent across individuals and measurements. For the histogram in Figure 4D
279 we used nine different TEM images from multiple sample fruits.

280 **Method Details**

281 **Optical Techniques**

282 For macro-spectroscopy (Figure 1C), deuterium - halogen tungsten lamp (DH-2000-BAL UV-VIS-NIR,
283 Ocean Optics) illumination by a double ended fibre (QR400-7-UV-VIS, Ocean Optics) coupled to a
284 Flame-S-UV-VIS miniature spectrometer was accessed by (Ocean Optics) software, and calibrated to
285 a white standard (WS-1-SL, Ocean Optics) and a mirror reflectance tile (Avantes RS-2) for total
286 intensity. The probe aperture was locked into a black aluminium mounting block obscuring stray
287 light, 1cm from the fruit surface.

288 *In situ* macro-spectroscopy of fruits and leaves (Figure S3D) was performed with an Ocean Optics
289 USB2000 spectrometer illuminated by deuterium and halogen lamps, standardized with a Spectralon
290 white reflectance standard (Ocean Optics, Dunedin, FL). Reflectance was measured 3mm from the
291 surface of the fruit or leaf.

292 For the optical microscopy (Figure 1B, S2-S3) and micro-spectroscopy (S2-S3), a Zeiss A1 microscope
293 was used in reflection mode for optical measurements with a Zeiss Hal100 halogen lamp and a
294 water-immersion objective lens (Zeiss W N-Achroplan 10x, 40x and 63x magnification). The image
295 was captured on a CCD (IDS UI-3580LE-C-HQ) and accessed by uEye Cockpit user interface software.
296 For polarisation measurements, incident (Zeiss 427710-9000) and reflection (Thorlabs 25 mm wire
297 grid) polarisers were used for polarisation control. For micro-spectrometry, the reflected light was
298 coupled via optical fibre (Avantes 50µm internal diameter) to a spectrometer (Avantes AvaSpec-
299 HS2048) and calibrated to a silver mirror (Thorlabs, PF10-03-P01).

300 The high magnification macroscale image in Figure S1A was taken using built-in LED full-ring
301 illumination of a VHX-5000 Keyence digital microscope.

302 Photo in Figure 1A was taken with a Sony DSLR-α300 and SAL30M28 macrolens. The UV-only photo
303 Figure S1B was taken with a Nikon DSLR-D90 camera, 105mm f4.5 UV-Micro-APO lens (Coastal
304 Optics) and Baader U-Filter 60nmHBW/320-380nm.

305 **Electron Microscopy**

306 Cryo-electron microscopy was carried out on fresh fruits sectioned, mounted on carbon glue and
307 flash frozen by infiltration with liquid ethane at -195°C and coated with 5 nm platinum using an FEI
308 Varios 460 microscope with Quorum PP3010T cryo-SEM preparation and transfer system at
309 Cambridge Advanced Imaging Centre.

310 For TEM, fresh tissue was prepared by immersion in Karnovsky's fixative for 1-14 days, dissected,
311 and stained with osmium tetroxide for 2 hours. Tissue samples then experienced graduated
312 infiltration with an ethanol-resin series to fixation in LR White resin and polymerised for 24 hours in
313 vacuum at 60°C . [41] A Leica UCT microtome and diamond knife was used to cut 70-100 nm sections.
314 Sections were imaged on a Hitachi H-7650 transmission electron microscope (TEM) and a JEM 2100
315 Plus (Jeol) equipped with a RIO 16 CMOS camera (Thermo Fisher Scientific).

316 For serial tomography, serial semi-thick (300 nm) sections were cut using a Leica UCT umicrotome
317 and diamond knife and mounted on a Formvar-coated slot grid. 15nm-diameter colloidal gold
318 particles were attached to both sides of the grids to serve as fiducial markers in the tomographic
319 reconstructions. Series of tilted images from -60° to 60° angles were recorded every 1° using a
320 TECNAI F30 (FEI) operated at 300kV. Images from three sections were combined into a single, serial
321 tomogram using the IMOD software package [42] and the lipid surfaces modelled using the IMOD
322 isosurface function.

323 **Staining Histology**

324 Staining of the ultrathin sections of embedded samples was used for identification of structural
325 composition in light microscopy (Nile Blue A, Sudan Black B) and electron microscopy (imidazole
326 buffer, ruthenium red). Nile Blue A stains neutral fats and waxes a red or pink colour but fatty acids
327 blue [24] (Figure 3C). Sudan Black B stains lipids a dark blue or black colour (Figure S2D). [17]; [43]
328 Stains were applied for 60 minutes at room temperature (Nile Blue A, after washing in acetic acid for
329 10s and isopropanol for 30s) or 60°C (Sudan Black B). Staining with Oil Red O was attempted but
330 failed to penetrate the resin.

331 For chloroform extraction, transverse ultrathin sections of epicarp with a thickness of 80-90 nm
332 were cut at cryogenic temperature (-110°C) with a diamond knife on a Leica EM UC7
333 ultramicrotome and collected on carbon-coated copper grids. Grids with cryo-sections were exposed
334 to chloroform under reflux for 1 hour. Both untreated and treated sections were then observed
335 without further post staining using a TEM (JEM 2100Plus, JEOL) equipped with RIO16 CMOS camera
336 (Gatan Inc.) and operated at 200 kV.

337 **Electron Diffraction**

338 Selected area electron diffraction (SAED) experiments were carried out with untreated cryo-sections
339 and a TEM (JEM 2100 Plus) operated at 200 kV. A selected area aperture with a diameter of 500 nm
340 was used. The SAED patterns were recorded on the RIO16 camera using the low-dose module of
341 Serial EM software.

342 **Structural Analysis**

343 TEM profiles were analysed using Fiji [44] to extract the dimensional statistics of the two different
344 refractive index layers. Nine globular multilayer cross-sectional TEM images were binarised and
345 image intensity profiles measured, from which the width distributions of cellulose and lipid layers
346 were taken.

347 **Numerical modelling**

348 2D structures with different types of disorder were generated using a recently developed inverse
349 design algorithm discussed in detail in Jacucci et al.[26] Numerical simulations of the optical
350 response of the generated structures were performed in Lumerical, a software using the finite
351 difference time domain (FDTD) method. The simulations were performed using periodic boundary
352 conditions in the direction perpendicular to the incoming beam – set as a plane wave – and perfect
353 matching layer (PML) boundaries in the thickness direction. The numerical stability/convergence of
354 the simulations was ensured by choosing an extensive simulated time of 0.1ns and boundary
355 conditions as stated. This ensured that the electric field in the structure decayed before the end of
356 the calculation and that all the excitation light was either reflected or transmitted. Each of the
357 presented curves was obtained averaging the optical simulations of five different ensembles of
358 particles with identical parameters.

359 The disordered 1D multilayer was built with the `jreftran`[45] function in MATLAB. An average of 100
360 trials were taken of 1D multilayer stacks of 160 pitch repeats, with lipid and cellulose layers having
361 widths 58 and 67 nm respectively, with a standard deviation of 25 nm and refractive indices $n = 1.47$
362 and 1.55 respectively, taken from literature values as described in the main text. The upper
363 refractive index boundary was matched to non-absorptive water ($n=1.33$) and the lower to a
364 complex refractive with light absorption taken from measurements of cyanidin-3-glucoside extracted
365 from bilberry (*Vaccinium myrtillus*).[29] Angle of incidence was 0° .

366

367 **Quantification and Additional Analysis**

368

369 No further methods were used to determine whether the data met assumptions, as a statistical
370 approach was not taken.

371

372 **Additional Resources**

373

374 **Video S1: Movie showing 3D rotation of globular multilayer structure model. Related to Figure 2D.**
375 Video reconstructed from three sets of TEM serial tomography using the isosurface function in iMod.
376 The single view shown in Figure 2D corresponds to a frame of this reconstruction.

377 This study did not generate any unique datasets or code.

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