Supporting Information

"3D Photoluminescent Lanthanide-doped Barium Titanate Structures Synthesized by Coating and Shape-preserving Reaction of Complex-shaped Bioorganic Templates," by Jonathan P. Vernon, Nicholas Hobbs, Ye Cai, Alfred Lethbridge, Peter Vukusic, Dimitri D. Deheyn, and Kenneth H. Sandhage

Detailed processing methodology

Papilio blumei butterfly scales (insect-sale.com, Chia-yi city, Taiwan) were chemically transformed into barium titanate ($BaTiO_3$, BT) and Eu-doped $BaTiO_3$ (Eu-doped BT) replicas via a three-step process: (i) application of a thin Ti-O-bearing coating through a layer-by-layer (LbL) surface sol-gel (SSG) process, (ii) pyrolysis of the underlying chitinous template and crystallization of the titania coating, and (iii) conversion of the resulting titania structure into BT or Eu-doped BT by microwave hydrothermal (MWHT) reaction with a barium acetate-bearing or barium-and-europium-acetate-bearing solution. For the first step, a 1 cm x 1.5 cm blue-green section of a *P. blumei* forewing was clamped (dorsal side up) to a silicon wafer and then immersed in a solution of 25 mM titanium(IV) isopropoxide (99.999% purity by metals basis, Sigma Aldrich, St. Louis, MO USA) in anhydrous isopropyl alcohol (IPA, Acros Organics, Geel, Belgium) for 2 min. After rinsing three times with 4 mL of IPA, the wing section was immersed in a solution of 60 vol.% deionized (DI) water in IPA for 2 min. The wing scales were rinsed again three times with 4 mL of IPA followed by drying with warm (28°C) flowing air for 5 min. This process (alkoxide exposure, IPA wash, DI water exposure, IPA wash, drying) was repeated for a total of 50 cycles. For the second step, the coated wing sections were clamped (Staples® black metal mini binder clips, Atlanta, GA USA) between two ground and polished (2.54 cm x 2.54 cm x 1.59 mm) fused quartz plates (Technical Glass Products, Painesville Twp., OH USA). The clamped assembly was heated at 0.5°C/min to 450°C, and then held at this temperature for 4 h in air to allow for pyrolysis of the chitin and crystallization of the titania. After cooling to RT, the resulting titania structures were sealed inside a 100 mL Teflon™ vessel (XP1500 Plus, CEM Corp., Matthews, NC USA) along with 20 mL of an aqueous solution of 1 M NaOH (in previously-boiled water) and either 125 mM barium acetate (99.9% purity, Sigma Aldrich) or 119 mM barium acetate with 6.3 mM europium acetate hydrate (99.9% purity, Sigma Aldrich). The molar reactant Ba:Ti or (Ba+Eu):Ti ratio within the vessels was ~200:1. The sealed containers were placed within a MWHT reaction system (MARS 230/60, 2.45 GHz, CEM Corp.) and heated within 5 min to 140°C (at a maximum power setting of 800W) and held at this temperature for 10 h. The resulting converted scales were separated from the reaction solution via centrifugation. The converted scales were then immersed in 25 mL of a 5 M acetic acid solution (diluted from glacial acetic acid, 17.4N, 99.9% purity, Fisher-Scientific, Pittsburgh, PA USA) in 50 mL centrifuge tubes (Corning, Lowell, MA USA) rotated at 30 rpm on a rotator (RKVSD, Appropriate Technical Resources, Laurel, MD USA) for 24 h to remove any $BaCO_3$ precipitates that may have formed upon opening the vessel in air or $Eu(OH)_3$ that may have formed during the hydrothermal reaction. The specimens were then rinsed twice with 20 mL of water, and then twice with 20 mL of IPA, followed by drying in air at 70°C for 1 h. Each rinse step was carried out via 5 min centrifugation of solutions in

50 mL centrifuge tubes at 4,500 rpm in a centrifuge equipped with a swinging bucket rotor (5408R centrifuge, #A-4-44 rotor, Eppendorf, Hamburg, Germany), followed by removal of supernatant before adding a subsequent solution or drying. Deposition of photoluminescent Eu-doped BT scales onto filter paper was conducted by placing 0.1 mL of a 1 mg/mL suspension of butterfly scales in IPA solution into a 2.0 mL Spin-x® centrifuge tube filter (Corning, Inc., Corning, NY USA) lined with filter paper (Qualitative P5 medium porosity grade, Fisher Scientific). The solution was filtered through centrifugation (5415D centrifuge, Eppendorf) at 13,200 rpm for 1 min and then dried at 70°C for 1 h.

Specimen characterization

A photograph of a native (non-living) *Papilio blumei* butterfly was taken with a 7.1 mega pixel PowerShot A710 IS digital camera (Canon USA, Inc., New Hyde Park, NY USA) with the macro feature and image stabilizer enabled (no flash). Transmission and fluorescence optical images of blue-green *P. blumei* scales and scale replicas were obtained with a UV confocal microscope (LSM 510, Carl Zeiss MicroImaging, LLC, Thornwood, NY USA). Transmission optical images were obtained with the use of a 543 nm (1 mW He-Ne) laser. Fluorescence images were obtained with the use of 488 nm (30 mW Ar-ion) laser excitation and a long pass 585 nm filter through a 10X objective (Plan-neofluor, NA=0.3). For qualitative comparison of relative fluorescent intensity, images were generated in the same session using the "Re-Use" function in the ZEN 2009LE software ensuring identical optical path and imaging conditions (i.e., the same gain, laser power, pin hole, brightness, and contrast settings). Bright field and dark field images were obtained with a Keyence VHX-600 digital microscope (Keyence Corporation, Osaka, Japan) outfitted with 250X - 2,500X objectives. Before obtaining such images, the white balance was set using a Teflon™ block in bright field mode. All bright and dark field micrographs were generated with a minimum of 5 sequential frames taken at decreasing z-height. For bright field, dark field, and fluorescence imaging, *P. blumei* scale or scale replica specimens dispersed in IPA were deposited onto pre-cleaned (2.54 cm x 7.62 cm x 1 mm) Gold Seal® micro slides (Gold Seal® Products, Portsmouth, NH USA) with a 1 mL pipet and then allowed to dry. Dried filter paper specimens containing Eu-doped BT replica scales were also placed on such glass micro slides. The specimen-bearing micro slides were then covered with a square (2.2 cm x 2.2 cm) cover glass slip (No. 1.5, VWR International, LLC, Radnor, PA USA). For each specimen, the cover slip was secured to the glass slide via nail polish (Continuous Treatment Strength, Sally Hansen®, Coty, Inc., New York, NY USA).

The morphologies of the starting blue-green *P. blumei* wing scales, the SSG coated scales, the pyrolyzed scales, and the MWHT reacted scales were evaluated with a field emission scanning electron microscope (1530 SEM, LEO/Carl Zeiss) after mounting the scales or scale replicas directly on aluminum stubs (Ted Pella, Inc., Redding, CA USA).

Specimens for X-ray diffraction (XRD) analyses were dispersed in IPA, deposited with a pipet onto a low background quartz silica substrate (cut 6° from (0001), The GEM Dugout, State College, PA USA), and then allowed to dry. XRD analyses were conducted on an X'Pert Pro Alpha-1 diffractomer (PANalytical B.V., Almelo, Netherlands) with monochromatic Cu $K_{\alpha 1}$ radiation emanating from a 1.8 kW X-ray tube (45 kV, 40 mA) through a symmetrical Johannson monochromator. The source was outfitted with 0.04 rad soller slits, a 1° fixed anti-scatter slit, a ½° programmable divergence slit, and a 10

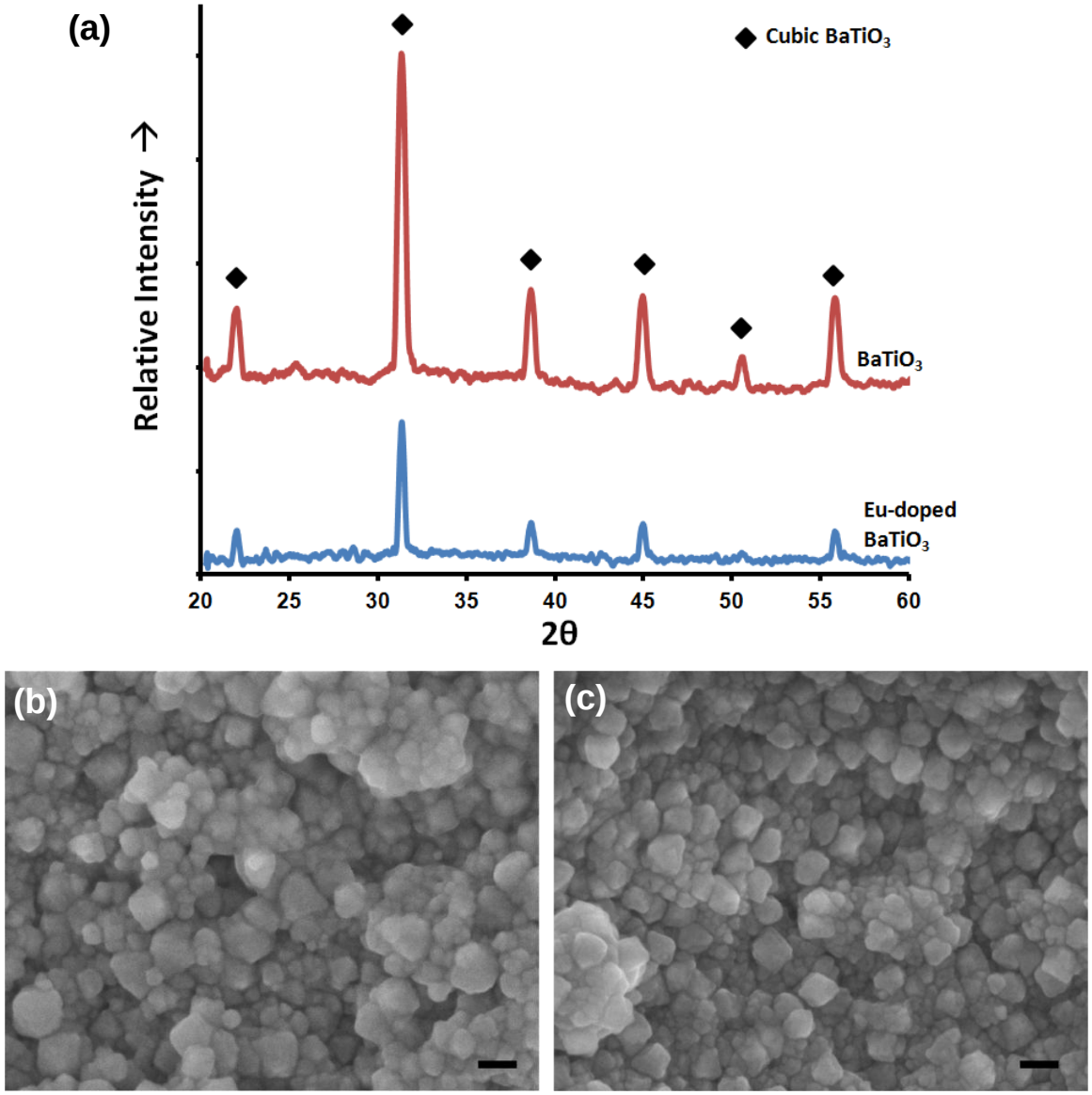
mm mask. The diffracted beam optics were outfitted with a 5.5 mm anti-scatter slit and 0.04 rad soller slits placed before the X'Celerator detector. Each pattern was generated by summing 4 identical 30 minute scans conducted with Bragg-Brentano geometry and a step size of 0.01671 degrees 2θ.

For laser ablation inductively-coupled plasma mass spectroscopic (LA-ICP-MS) analyses, the Eu-doped BT scale replicas were mounted on carbon tape. A 193 nm ArF excimer laser (UP193HE, New Wave Research, Fremont, CA USA) with beam homogenizing optics was used to ablate each sample using a single laser pulse (20 ns, 100 μm diameter beam at the sample, approximately 0.3 mJ laser pulse energy). A 1.0 L/min He gas flow carried the ablated products out of the cell for mixing with 1.0 L/min Ar before being introduced into the 1300 W inductively-coupled plasma. An inductively-coupled plasma sector field mass spectrometer (ThermoFinnigan Element 2, Bremen, Germany) was used to measure ^{138}Ba and ^{153}Eu signals with a mass spectral resolution (m/Δm) of approximately 300. ICP-MS signals were integrated over an approximately 10 s time period that a signal was observed following a single laser pulse. A glass standard[1] (SRM 610, National Institutes of Standards and Technology, Gaithersburg, MD USA) was used for calibration of the Ba and Eu signals.

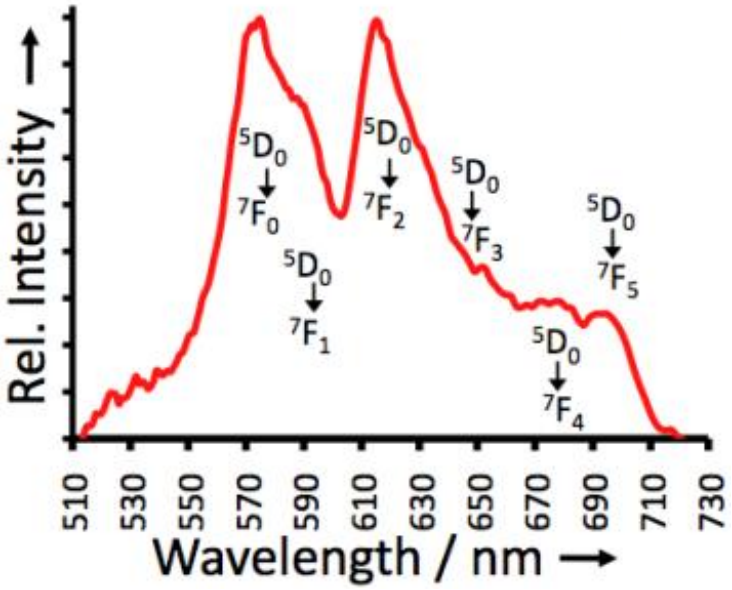
Photoluminescence spectra were collected with a hyperspectral imaging system (PARISS PHSI, LightForm, Inc., Asheville, NC USA). The system incorporated a prism-based spectrograph using a monochrome CCD camera (Retiga 2000R, QImaging, Surrey, BC Canada) to collect spectral information. The system was mounted on a Nikon E800 microscope (Nikon Instruments, Inc., Melville, NY, USA). Wavelength calibration was performed with a MIDL Hg+/Ar+ emission lamp (LightForm, Inc.). Wavelength accuracy was recorded and verified to be within 0.3 nm. A given specimen was immersed in IPA and held between a glass slide and cover glass slip. For a given specimen, 200 acquisitions, each of 2 sec duration, were obtained. The spectrum generated for a given specimen was based on the consensus of 98% of all collected spectra (>12,000 spectra) for that specimen.

[1]Pearce, N. J. C.; Perkins, W. T.; Westgate, J. A.; Gorton, M. P.; Jackson, S. E.; Neal, C. R.; Chenery, S. P. *Geostand. Newslett.* **1997**, *2*1, 115.

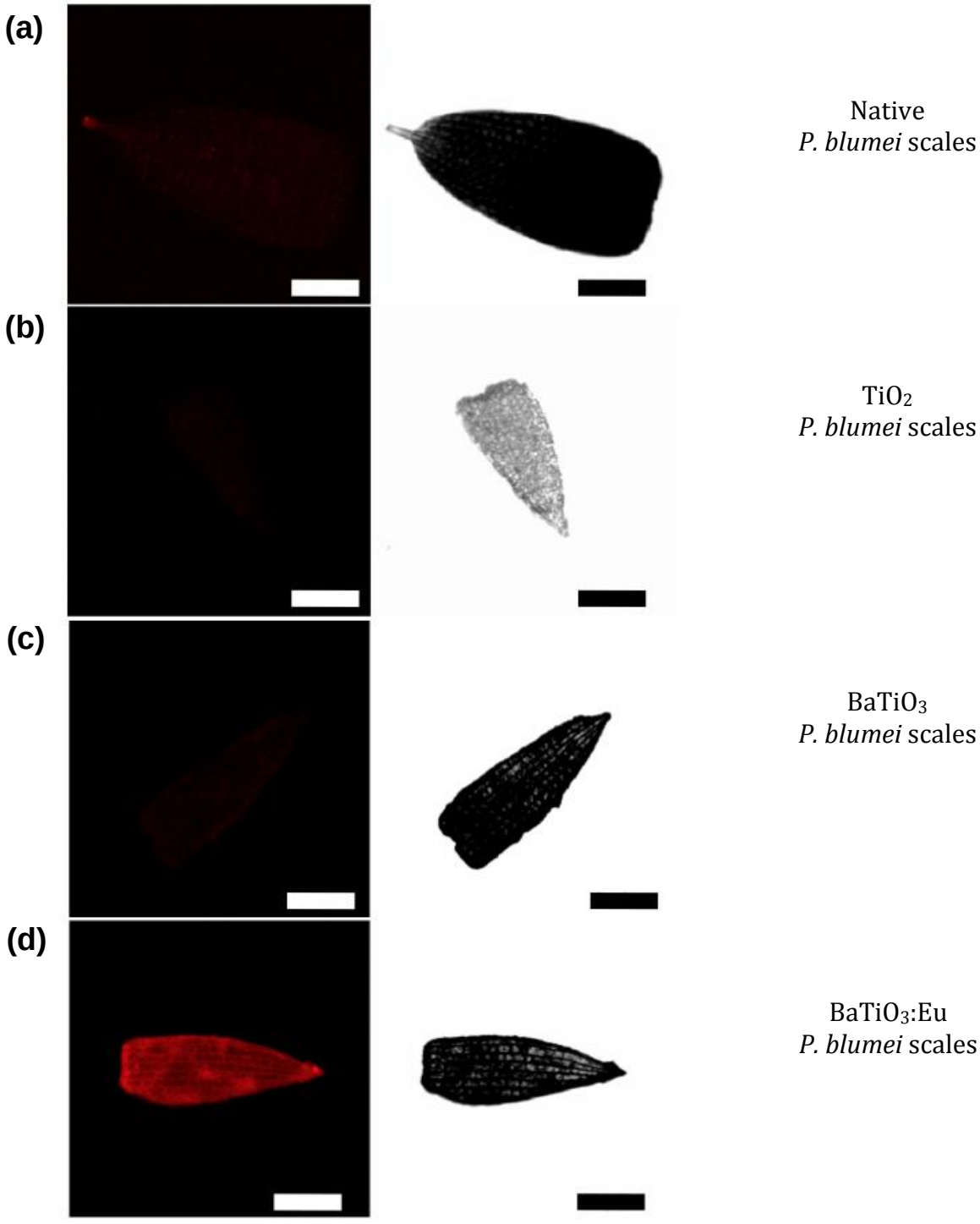
[2](a) Li, J.; Kuwabara, M. *Sci. Technol. Adv. Mater.* **2003**, *4*, 143. (b) Weatherspoon, M. R.; Haluska, M. S.; Cai, Y.; King, J. S.; Summers, C. J.; Snyder, R. L.; Sandhage, K. H. *J. Electrochem. Soc.* **2006**, *153*, H34. (c) Kishi, H.; Kohzu, N.; Sugino, J.; Ohsato, H.; Iguchi, Y.; Okuda, T. *J. Euro. Ceram. Soc.* **1999**, *19*, 1043. (d) Eylem, C.; Saghi-Szabo, G.; Chen, B.-H.; Eichhorn, B.; Peng, J.-L.; Greene, R.; Salamanca-Riba, L.; Nahm, S. *Chem. Mater.* **1992**, *4*, 1038.



Supplemental Figure 1. (a) XRD analyses of $BaTiO_3$-converted and Eu-doped $BaTiO_3$-converted *P. blumei* butterfly scales. Peak positions and intensities were matched to Powder Diffraction File Card No. 04-007-6869, which has the highest quality mark (Indexed) in the International Center for Diffraction Data database for cubic ($Pm\bar{3}m$) $BaTiO_3$. SE images of nanoparticles present on (b) the $BaTiO_3$ scale replica surface and (c) the Eu-doped $BaTiO_3$ scale replica surface. Scale bars = 100 nm.



Supplemental Figure 2. A consensus photoluminescence emission spectrum (generated via hyperspectral imaging with 436 nm excitation and a 510 nm long pass emission filter) obtained from a Eu-doped $BaTiO_3$ *P. blumei* scale replica. The positions of intra-4f-shell electronic transitions for Eu^{+3} are shown.[2]



Supplemental Figure 3. Confocal fluorescence (left column) and transmission (right column) micrographs of: (a) a native *P. blumei* scale (top row), (b) a TiO_2 scale replica (after LbL SSG coating and organic pyrolysis, second row), (c) a $BaTiO_3$ scale replica (after MWHT reaction, third row), and (d) a Eu-doped $BaTiO_3$ scale replica (after MWHT reaction, bottom row). All scale bars = 50 μm.